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UTILIZATION OF DRIED EGG WHITES IN BAKERY PRODUCTS

BETTY M. WATTS and CATHERINE ELLIOTT

Department of Home Economics, University of California, Berkeley, California

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In spite of the relative economy with which dried whites can be produced, transported, and stored, their consumption remains insignificant as compared with that of frozen whites. In fact the use of dried whites is tending to become restricted to certain types of confections, meringues, and prepared pudding and flour mixtures where the dried material has no competitor (Le Clerc and Baily, 1940; Termohlen, Warren, and Warren, 1938). The baking industry, largest single commercial consumer of egg products, makes little use of dried whites.

Much evidence has been accumulated to show that commercial dried whites are definitely inferior to fresh or frozen whites in many bakery products (Balls and Swenson, 1933; Bailey and Le Clerc, 1935). This is confirmed in the present article. However, the evidence here presented indicates that the poorer performance of the commerical dried whites is caused by the various treatments to which the whites are subjected before drying.

The purpose of these treatments has been stated to be, first, to liquefy the white so that it will be thin and homogeneous, and second, to increase the volume and stability of the foam which may be produced from the reconstituted dried white. Several methods may be used to accomplish these results:

1. The whites may be allowed to ferment in open vats from four to six days. During this period enzymes within the white itself, plus proteolytic microörganisms, cause liquefaction of the white. The dried material imported from China is of this type.

2. Tryptic enzymes may be added to hasten the fermentation and bring about the desired results in less time and with a lower bacterial count (Balls and Swenson, 1936).

3. The whites may be treated with strong acid before drying (McCharles and Mulvaney, 1937; Fischer, 1935).

The partial hydrolysis of egg albumin, as well as of other naturally occurring proteins, undoubtedly increases foaming ability. Heuser (1930) obtained a very active foaming material by heating egg white with strong bases. He states that the foaming agent is probably a metaprotein or proteose, and that the increased foaming may be brought about by acids and enzymes as well as by bases.

However, to what extent such changes in the natural egg proteins are beneficial in food preparation remains to be demonstrated. Simple meringue mixtures might be expected to be improved by the increased whipping ability. But in baked products, even where foaming capacity is admittedly important (angel and sponge cakes), proper extensibility and coagulability of the protein structure are equally important. These latter qualities become the main consideration when egg albumin is used in cakes containing fat, cream puff shells, etc., where the albumin is not whipped.

Experimental

In the present paper, the characteristics and performance in food preparation of the following types of albumin are compared:

1. Fresh white, obtained from U. S. Specials bought at a local market.

2. Laboratory-dried white, prepared from the fresh by drying on porcelain plates at 45°C. in a vacuum oven. The drying was completed in 6 to 10 hours. The partial vacuum caused the liquid to froth slightly, so that the dried material was porous in texture and easily scraped from the plate. No treatment of any kind was given the eggs before drying.

3. Fermented flake albumin obtained from a recent shipment from Shanghai.

4. A commercial dried white prepared in this country by acid treatment followed by spray drying (Fischer, 1935).

Except where otherwise stated, the dried albumins were made up in the form of homogeneous 13% solutions with distilled water for comparison with fresh white.

The Chinese fermented sample had a distinctively strong and unpleasant odor. This odor was apparent in all products made from the Chinese white. Although it could be covered up by using excessive amounts of flavoring, it nevertheless constitutes a definite objection to the use of fermented material. All other samples were practically odorless.

Other characteristics are shown in Table I. The longer time required to reconstitute the laboratory vacuum-dried and the Chinese fermented samples was at least partly due to the flake form in which

they are dried. Pulverization would undoubtedly shorten the time necessary for solution.

TABLE I

PHYSICAL CHARACTERISTICS OF EGG WHITE SAMPLES

Sample	Approximate time to dissolve	Consistency	Turbidity	pH	Maximum foam volume as % original liquid	Time to reach maximum foam	Drainage in 30 minutes
	Hrs.				%	*Min.*	*Ml.*
Fresh white	—	Characteristically viscous	Clear	8.6–9.0	1100	7	6.0
Vacuum-dried without treatment	3	Thick white, retains structure and viscosity	Clear	9.6	1050	7	5.5
Chinese fermented	1½	Watery	Somewhat turbid Suspended matter	6.0	1200	5½	2.0
Acid-treated, spray-dried	½	Watery	Very turbid	4.6	1230	5½	0.1

The watery consistency of the commercial dried whites is a result of the treatment before drying. The structure of the thick white is completely broken down during this treatment. This reduction in viscosity appears to be one of the aims of the treatment, since it has been shown that thin white whips more readily than thick (Bailey and Le Clerc, 1935).

The turbidity of the commercial dried whites is correlated with their low pH. The pH of the acid-treated sample is identical with the isoelectric point of egg albumin. Egg white dried without treatment, on the other hand, shows a higher pH than the original fresh material, due presumably to loss of carbon dioxide during the drying. (Values below pH 8.0 were measured on a quinhydrone electrode; above pH 8.0 colorimetric methods were used.)

As might be expected, the treated samples whipped better than the fresh or vacuum-dried. Not only was the foam volume greater, but also maximum volume was obtained in less time and the foams were much more stable, as evidenced by the smaller amount of drainage. (In each case 30 g. of reconstituted white was whipped in a quart bowl with an electric mixer. The foam was transferred to measuring cups with the least possible handling, and the cups inverted in funnels draining into graduated cylinders. Whipping time was varied from

3 to 8 minutes to obtain the maximum foam volume for each kind of white.)

Meringues

To test their performance in simple meringue mixtures, three typical products were prepared from each sample, as follows:

1. Simple, uncooked frosting, prepared by beating confectioner's sugar into the white. (Thirty g. of white was whipped for 2 minutes; 50 g. of sugar was then added while beating continued over a period of 4 minutes. The volumes of the resulting stiff meringues were determined by packing in measuring cups.)

2. Boiled icing, also known as marshmallow, divinity, or heavy meringue icing, prepared by beating a hot sugar syrup slowly into whipped egg white. (Thirty g. of white was whipped 3 minutes. A hot syrup, consisting of 150 g. of sugar, 10 g. of corn syrup, and 60 ml. of water, boiled to 15°C. above the boiling point of water, was poured into the whites while beating continued over a period of 5 minutes. Volume of the icing was measured as above.)

3. Baked meringues (meringue shells or kisses) for which a whipped egg white and sugar mixture is permanently set by baking in a slow oven. (To 30 g. of egg white beaten for 2 minutes, 50 g. of powdered sugar was added during beating over a period of 3 minutes. Fifteen-gram portions of the meringue were weighed onto baking sheets and baked at 275°F. for 45 minutes.)

It is to be noted that all of these products consist essentially of a whipped egg white and sugar mixture. Neither flour nor fat is used. Extensibility and heat coagulability of the protein framework are probably of minor importance even in the baked meringues, since the baking process is mainly a slow drying out.

Under these conditions the commercial treated whites were superior to the fresh or plain dried. The volumes of frosting obtained (Table II) were greater with the treated whites and the boiled icings were stiffer, maintaining peaks indefinitely. This was also true when the initial whipping period (before addition of sugar) was increased to five minutes.

TABLE II

VOLUME OF FROSTING

Egg sample	Volume of cold meringue	Volume of boiled meringue
	cc.	*cc.*
Fresh	180	300
Vacuum-dried	190	315
Chinese fermented	240	375
Acid-treated	230	380

Of the baked meringues, those from Chinese fermented white were largest, very white in color, with a compact marshmallow-like internal texture. The acid-treated were similar to the Chinese but slightly smaller in size. The fresh and vacuum-dried (practically indistinguishable) were smaller, light tan in color, and partially hollow, with a light, moist internal texture.

Batter Products

Cream-puff shells and *popovers* are particularly well adapted to a study of the baking properties of egg white, since in both products the white is essential as a source of extensible protein and the manipulation of the batter is relatively unimportant as a variable.

For both products, standard batters were prepared containing all ingredients except the egg white. The batters were then weighed out into four separate portions and one of the four types of white added to each portion. After thorough incorporation of the white, equal portions of the completed batters were weighed onto baking tins.

The commercial treated whites produced cream-puff shells less than two-thirds the size of those from fresh white (Fig. 1), and were

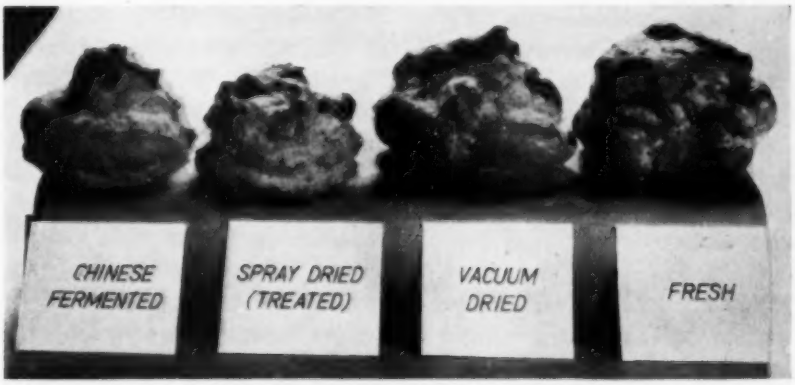

Fig. 1. Comparison of whites in cream puffs.

poorly browned and heavy walled. These shells were only slightly better than shells from which white was omitted entirely. The sample dried without treatment, on the other hand, produced shells only very slightly inferior to fresh, nicely browned shells, with thin translucent walls.

Similar but even more marked results were obtained with the popovers. Treated samples produced popovers of less than half the regular size; that is, they failed to pop. The top crust usually cracked during baking with eruption of contents and loss of steam. Popovers

from vacuum-dried whites were equal in all respects to those from the fresh material.

In *butter cakes*, the poorer baking qualities of the treated dried whites become more apparent the richer the mix, *i.e.*, the more egg, fat, and sugar for a given amount of flour. This might be expected, as the leaner formulas (approaching muffin mixes) depend mainly upon gluten development for their structural framework. Egg white may be omitted entirely with no serious effect. In the richer cakes, where the egg is essential, none of the dried whites were entirely equal to the fresh. However, the untreated vacuum-dried were a close second to the fresh in volume and texture, whereas the treated whites both produced cakes of considerably less volume with soggy streaks near the bottom.

Standard white cakes from the four types of white differed mainly in color of crust. Fresh and vacuum-dried white produced cakes with well browned crusts, whereas cakes from commercial samples were light in color as well as smaller in volume. No differences in texture were noted.

For *cakes without fat* (sponge and angel food), a whipped sugar and egg meringue that will stand up upon the addition of flour is essential, as well as proper elasticity and coagulability of the cell structure. Pyke and Johnson (1940) suggest that moderately stable meringues which drain fairly rapidly are preferable to very stable meringues with little drainage, since flour particles can be incorporated in the former in pockets between bubbles, thus contributing to the structural framework of the cake. With very stable, non-draining foams, on the other hand, the addition of the flour causes foam collapse, since only upon collapse of the bubbles is liquid made available for the flour.

The results obtained with the dried whites on angel cake support this view. The high flour and sugar formula recommended by Barmore (1936) was used, and also the method of mixing which he recommends as giving the best volume, *i.e.*, beating all the sugar with the egg white before addition of flour. The flour was folded by hand. It was noted during this folding process that the acid-treated white (most stable of the foams) was broken down more upon addition of flour than any of the other meringues. The resulting cake was smaller in volume than any of the others, with a heavy compact texture. The other three cakes were identical in volume, but the fermented white produced a somewhat harsher texture than the fresh and untreated.

Excellent whole-egg sponge cakes were obtained from fresh and untreated whites, using the single meringue method recommended by Pyke and Johnson (1940), whereas cakes containing the treated whites

were complete failures, about one-third normal volume, heavy and rubbery. This failure was due to the fact that a stable meringue could not be obtained when the treated whites were mixed with yolk. Additional evidence of the marked depressing effect of yolk on the treated whites is shown in Table III.

TABLE III

VOLUME AND STABILITY OF EGG WHITE FOAMS IN THE PRESENCE OF EGG YOLK

In each case 3 g. of fresh yolk was added to 40 g. of fresh or reconstituted white and the mixture whipped 6 minutes.

Sample	Increase on whipping		Drainage in 30 minutes
	%		
Fresh	320		3.5
Vacuum-dried	330		5.0
Chinese fermented	300	froth on liquid layer	28.0
Acid-treated	180		32.0

The serious effect of small amounts of yolk in destroying foaming of the dried whites has been traced to the low pH of the latter. When the pH of the acid-treated white was adjusted to 9.0 by addition of sodium hydroxide to the water used for reconstituting, a volume increase of 470% was obtained on whipping with yolk and no drainage was obtained. Barmore (1936) mentions the fact that egg yolk reduces the foaming of fresh egg in acid solutions much more than at the original pH of the white.

However, the generally poorer baking qualities of the treated samples of dried white cannot be ascribed entirely to their low pH. The only product which was greatly improved by the use of the alkalized white was the whole-egg sponge cake, which was equal in volume and texture to the fresh and vacuum dried. Cream puff batter was thicker with alkalized than with the regular treated white, resulting in a more knobby appearance to the baked puff, but the volume was not improved. Popovers showed little or no improvement. Whipping volume was greatly decreased (800% as compared to 1230%) so that the alkalized material would be unsuitable for those products (meringues, confections, etc.) where the treated whites were found to be advantageous.

Probably a lack of heat coagulable protein, owing to partial hydrolysis of the acid-treated and fermented materials, is the chief cause of their failure in baking. Increasing the amount of dried white used in cream puffs from 13% to 20% produced a puff comparable in volume to that from fresh white, provided the pH of the white was adjusted to 9.0. Increasing the concentration of dried material without adjust-

ing the pH caused extreme thinning of the batter, so that the resulting puffs were smooth and flat, without the characteristic knobbiness of shells from fresh white.

Discussion

This work demonstrates that dried white can be used almost, if not quite, as successfully as fresh white for general baking purposes, provided white from good-quality eggs is dried quickly at a temperature below the coagulation point, and without previous treatment that has caused denaturation of the protein.

From a practical standpoint it would be desirable to have a simple test by which the baking qualities of commercial samples of dried white could be rated. Lacking suitable physical or chemical tests, it is suggested that a popover baking test would probably give the needed information most quickly and certainly, and with little expenditure of materials.

Sufficient batter, including all ingredients except the white, should be prepared for all the samples of white to be tested. The formula adopted in this laboratory is as follows:

All purpose flour	100 g.
Water	200 g.
Egg yolk	34 g.
Salt	2 g.

To each 100 g. of batter (mixed until smooth) is added 30 g. of one of the whites to be tested. (A control is conveniently run on another 100-g. portion of the same batter, using 30 g. of fresh white.) After thorough mixing of the white, 20-g. portions of the completed batter are weighed into muffin tins and baked for 20 minutes at 450°C. and another 20 minutes at 350°C.

A number of commercial dried whites have been tested in this way. Some of these had obviously been fermented. The history of other samples was not known. No material known to be enzyme treated could be obtained. All of the commercial samples tried produced very poor popovers, similar to those made from the acid-treated and Chinese fermented and far inferior to the samples dried in this laboratory.

Obviously, partially hydrolyzed, dried material with high whipping ability has an accepted place in the preparation of various types of meringue mixtures. A simple whipping test for foam volume and drainage is widely employed for testing dried whites to be used for this purpose, according to Le Clerc and Bailey (1940). This test is of course useless in determining the suitability of the white for baking. It is suggested that whites suitable for baking should be dried as a

separate article and carefully tested to see that they retain their baking characteristics.

Summary

Commercial samples of dried white were found to whip better and to be more suitable for meringue mixtures than fresh white or vacuum-dried material prepared in the laboratory. The increased whipping ability of the commercial whites is believed to be due to the partial hydrolysis brought about by special treatment (fermentation or acid treatment) given them before drying.

On the other hand, in all batter and dough products the commercial treated whites were greatly inferior to fresh or laboratory-dried whites. This inferiority was traced to their low pH and decreased amount of heat-coagulable protein.

Laboratory-dried (untreated) white was found to be only slightly inferior to fresh white for baking. A simple baking test to determine the suitability of dried whites for batter and dough products is proposed.

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STANDARDIZATION OF THE EVELYN PHOTOELECTRIC COLORIMETER FOR FLOUR PIGMENT DETERMINATIONS[1]

D. S. BINNINGTON, W. S. HUTCHINSON, and CHARLES G. FERRARI

General Mills, Inc., Research Laboratories, Minneapolis, Minnesota

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Determination of the pigment content of flour extracts presents a special type of problem due to the fact that two groups of pigments are present, differing rather widely in their spectral properties. Until recently, it has been generally considered that carotene constituted the principal yellow pigment of flour; the work of Markley and Bailey (1935 and 1935a), however, indicates that wheat and flour pigments are a highly complex mixture, containing alpha and beta carotenes, xanthophyll and its esters, tricin and similar flavone pigments, together with other unidentified yellow coloring matters of the flavone type. An examination of the spectral distribution curves for typical high-grade-flour extracts, however, suggests that the pigments present are substantially carotenoid in nature, and recent unpublished studies by the senior author indicate that the major carotenoid pigment is xanthophyll or its esters, rather than carotene itself. The flavone pigments appear to be closely associated with the bran, and therefore appear in increasing amounts in lower-grade flours.

The problem of estimating the pigment content of a flour extract is thus not a simple one and cannot be adequately solved by simple colorimetric means. Of the various methods proposed, the spectrophotometric procedure of Ferrari and Bailey (1929) most closely approximates the ideal, since the estimation of carotenoid pigment is based upon a measurement of the transmittancy of the extract for monochromatic light of a single wave length (4,358 A. U.) at which point the absorption is very high. Under such circumstances, the interfering effect of flavone pigments is reduced to a minimum, and the method, while not specific for either carotene or xanthophyll, gives an excellent indication of the *total* carotenoid pigment present. Unfortunately, the equipment required is expensive, and the method in general is better adapted to research investigations than to routine determinations. It represents, however, the fundamental standard procedure to which all other methods should be compared.

The simplified colorimetric procedure described by Geddes, Binnington, and Whiteside (1934) does not require elaborate apparatus, especially in view of the production in recent years of inexpensive

[1] Paper No. 20, Journal Series, General Mills, Inc., Research Laboratories.

mercury vapor lamps. No standardization other than the preparation of known potassium chromate solutions is required. It is, however, a visual procedure, and as such is subject to the limitations and inaccuracies inherent in all methods employing the eye as a detector of photometric balance.

In recent years various types of so-called photoelectric colorimeters have appeared upon the market, and a number of workers in the field of flour pigments have turned to these instruments as a means of increasing the precision and rapidity of their tests. Most such devices are designed primarily for use with standard colorimetric procedures where a white light source and filters transmitting relatively broad regions of the spectrum are adequate; such apparatus is not well adapted to flour pigment determinations, since the sensitivity of the instrument is not great enough to provide adequate galvanometer response when a sharp-cutting filter of low total light transmission is employed. The use of such filters is essential if the interfering effects of flavone pigments are to be minimized, and if they are not employed separate calibration curves must be prepared for each grade of flour examined.

The most serious objection to the use of photoelectric instruments resides in this question of standardization. In conventional colorimetric procedures, calibration curves can be readily prepared by developing a series of color standards with graded known amounts of a reference material, but such methods are not applicable to flour carotenoids. Some workers have employed beta-carotene as a standard, but this practice is open to serious question, since the composition and spectral characteristics of the standard and the unknown differ widely. Furthermore, the reliability of commercial beta-carotene as a standard is very doubtful unless it is carefully purified. Under such circumstances, the only reliable standards would appear to be a series of flour extracts whose pigment content had been previously determined by means of a spectrophotometer. As equipment for such a proceeding is not generally available, the utility of photoelectric methods would appear to be very limited, unless instruments could be obtained in which the agreement between duplicate models was sufficiently good to permit of constructing a permanent calibration curve. It is believed that the Evelyn (1936) Photoelectric Colorimeter [2] meets these requirements.

Since the Evelyn instrument was originally developed for biological studies and is not as well known to most workers as some other types, a brief description of the apparatus and its advantages for this particular type of problem is here presented. It is a direct-reading in-

[2] Manufactured by the Rubicon Co., 29 North Sixth St., Philadelphia, Pa.

strument employing an exceedingly sensitive and well-damped galvanometer in conjunction with a 6-volt lamp and a barrier-layer type of photocell, thus enabling the use of very low light intensities. Several advantages are manifest in such a system; the low intensity permits of working in the linear portion of the photocell-output curve, thus minimizing fatigue and hysteresis effects. Secondly, it enables the use of comparatively sharp-cutting filters, isolating fairly narrow spectral regions. Thirdly, the current consumption of the lamp is so small as to permit of using a storage battery for long periods of time without recharging and thus provides a very stable illuminating system free from the effects of line voltage variations or drift due to heavy battery drain. The use of filters is an integral part of the instrument, and it cannot be employed with white light alone; furthermore, the absorption cells are merely specially selected $7\times\frac{7}{8}$-inch test tubes, obviating the necessity of using fragile and expensive cells of special construction.

With the above points in view, the present study involving calibration with both naphtha+ethyl alcohol (93 : 7) and water-saturated butanol solvents was undertaken.

Experimental

To check the variation between duplicate instruments, a comparison was made between stock models available in this laboratory and

TABLE I

COMPARISON OF THE "APPARENT DENSITIES" (L) OF POTASSIUM CHROMATE SOLUTIONS OBTAINED WITH TWO EVELYN PHOTOELECTRIC COLORIMETERS

K_2CrO_4—mg. per 100 cc.	Apparent density (L)	
	Instrument No. 1	Instrument No. 2
0.92	—	.0166
1.36	—	.0246
1.81	—	.0351
1.94	.0398	—
2.26	—	.0434
2.70	—	.0518
3.14	—	.0617
3.88	.0757	—
4.91	—	.0982
5.36	—	.1051
5.80	—	.1149
5.82	.1177	—
6.24	—	.1235
6.68	—	.1308
7.75	.1487	—
8.00	—	.1534
11.64	.2164	—
12.50	—	.2291
15.52	.2819	—

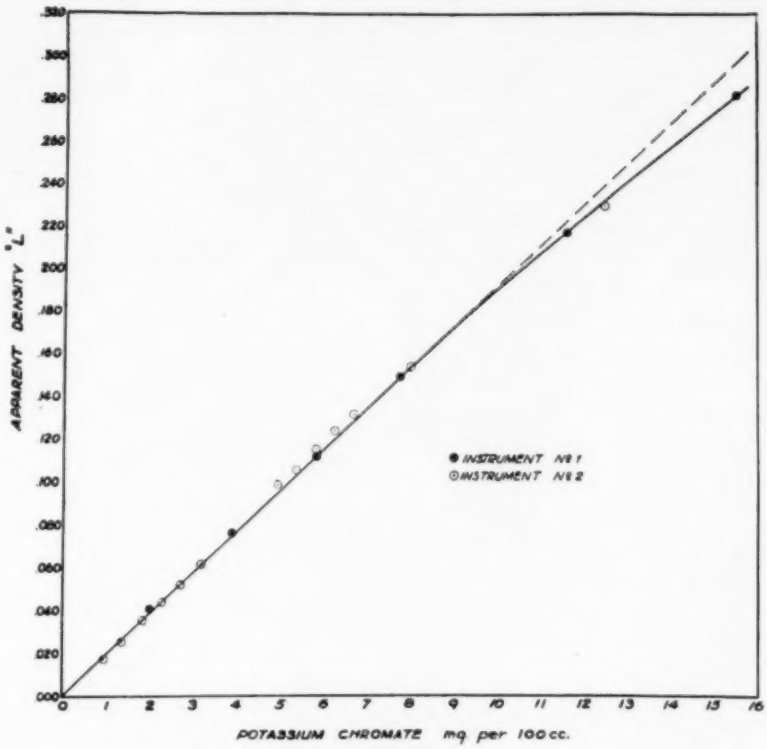

Fig. 1. Comparison of two Evelyn colorimeters.

in the research laboratory of the Ogilvie Flour Mills Co., Montreal, Canada. Both instruments were equipped with compound glass filters having a peak transmission of 440 millimicrons and a total transmission range of approximately 60 millimicrons. Because of the possibility of change in the transmittancy of flour extracts during shipment, the comparisons were made with dilute potassium chromate solutions prepared on the spot by each collaborator using the reagent-grade chemical, distilled water, and laboratory-grade glassware. Results of these comparisons are presented in Table I and graphically in Figure 1. It will be noted that the concentrations selected by the two operators were not identical and the best comparison is therefore shown in the graph. The tabulated data, however, should be of value in checking the uniformity of other instruments of this type. These results suggest that a high degree of uniformity exists between various instruments of this manufacture and therefore that a carefully worked out standardization for flour pigments should be of general utility.

General Technique of Standardization

The extracts employed in making the comparisons were freshly prepared, using both naphtha+absolute ethyl alcohol (93 parts to 7 parts) and water-saturated *n*-butyl alcohol. In the instance of naphtha+alcohol, the procedure described in *Cereal Laboratory Methods* (1935) was followed, involving a 20-g. sample of flour, 100 ml. of solvent, overnight extraction, and centrifuging. The flours employed were all commercially milled and included some first and second clears.

The butyl alcohol extracts were prepared with commercial butanol according to the procedure described by Binnington and Geddes (1939). The same sample-to-solvent ratio as with naphtha+alcohol was employed, with a 15-to-30-minute extraction time. Clarification was effected by filtration through No. 1 Whatman paper. The flours used in this group of tests were similar to those employed in the naphtha+alcohol study, with the addition of a number of experimentally milled unbleached samples supplied by the Division of Agricultural Biochemistry, University of Minnesota.

Pigment content of the extracts was determined with a König-Martens spectrophotometer, the transmittancies obtained being calculated into terms of carotene, using the values 1.91565 and 1.6632 for the specific transmissive indices of carotene in naphtha+alcohol and water-saturated *n*-butyl alcohol respectively. All readings were made at a wave length of 4,358 A. U. The spectrophotometric results are thus expressed in terms of carotene. It has been previously indicated that the pigments of wheat and flour are complex and expression of the transmittancy in terms of carotene can therefore only be considered as an approximation. Within the grades of flour examined, however, the spectral properties are essentially carotenoid, and the specific transmissive indices of carotene and xanthophyll are so close together that even if *all* the pigment were xanthophyll the error would only be in the order of 6%.

Readings with the Evelyn instrument were obtained by the following procedure: The illumination was turned on 30 minutes prior to making determinations to insure constancy of readings. A tube of the appropriate solvent was then introduced and the galvanometer deflection adjusted to a value of 100 on the scale. The solvent tube was then removed, the sample substituted, and the galvanometer reading noted. Such readings are numerically equivalent to percentage transmittancy, and when plotted yield a logarithmic curve. Accordingly they were converted to apparent densities by reference to a table supplied with the instrument. Density equals the negative logarithm of the transmittancy and a plot of such values is therefore linear over the region in which the solution obeys Beer's law.

TABLE II

STATISTICAL DATA FOR STANDARDIZATION OF THE EVELYN PHOTOELECTRIC COLORIMETER FOR CAROTENOID PIGMENT IN FLOUR EXTRACTS OBTAINED WITH NAPHTHA+ALCOHOL (93 : 7) SOLVENT IN THE INSTANCE OF 87 FLOURS

Correlation coefficient between spectrophotometric carotene (ppm. in flour) and apparent density (L) as determined with the Evelyn photoelectric colorimeter	$r = +.997$ (1% point = 0.275 approx.)
Regression equation: Carotene − ppm. (naphtha + alcohol solvent) = 0.046 + 0.1478 L (apparent density)	Standard error of estimate 0.048 ppm.

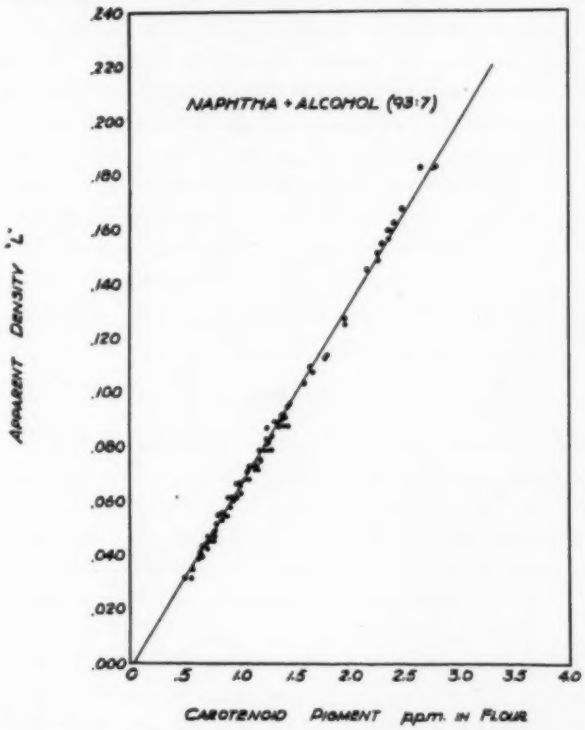

Fig. 2. Statistical data from 87 flours—naphtha+alcohol.

The results obtained were submitted to statistical analysis, and coefficients of correlation, regressions, and standard errors of estimate were computed. The results obtained with naphtha+alcohol are presented in Table II and the individual data together with the computed regression line are plotted in Figure 2. This regression line represents the calibration curve and may be used as such. It is preferable, however, to construct a table by listing the values of G (galvanometer

reading) in 0.25-unit increments, listing the corresponding values of L and computing the carotenoid pigment equivalents from the regression equation. Inspection of the plotted data indicates that close correspondence exists between spectrophotometric and photoelectric results, and the low standard error of estimate (0.048 ppm.) indicates that estimation of flour carotenoids may be made with high accuracy.

The results obtained with water-saturated butanol as a solvent are presented in Table III and the graphical relations illustrated in Figure 3. A greater deviation of the points from the regression line will be noted here than in the instance of naphtha+alcohol. This increased deviation is reflected in a larger error of estimate. However, the amount of pigment extracted by butanol is appreciably greater than in the instance of naphtha+alcohol, and this tends to reduce the difference when expressed on a percentage basis. The inclusion of additional data secured with a number of experimentally milled samples also has a tendency to cause a slight increase in the error of prediction. Although this error is approximately twice as great as the comparable value for naphtha+alcohol, both are so small as to be insignificant from a practical standpoint. Additional values are plotted in Figure 3, representing results obtained with 17 samples of ground durum semolina which were not included in the regression. These values fall very close to the regression line, indicating that this calibration may be employed in the analysis of durum semolinas.

Discussion

From the evidence presented, the Evelyn photoelectric colorimeter appears to be particularly well suited to the estimation of carotenoid pigments in flours. It is stable and extremely sensitive and may be equipped with suitable filters. In addition the two instruments of this manufacture examined are in sufficiently close agreement to justify the use of a calibration curve established with a single instrument. This last is a very important point, since one of the major objections to photoelectric colorimeters for this class of work is the difficulty of standardization, which ordinarily can only be satisfactorily effected if a spectrophotometer is available. However, the pigment calibration made in this study may be applied to other instruments of the same manufacture if it has previously been found that calibration of the instrument in question against potassium chromate standards agrees with the comparable data presented in this paper.

In the standardization of this instrument with extracts prepared with naphtha+alcohol and butyl alcohol respectively, the results obtained with the latter solvent show a wider scatter than the former. As the photoelectric results are secured by measurements obtained with

TABLE III

STATISTICAL DATA FOR STANDARDIZATION OF THE EVELYN PHOTOELECTRIC COLORIMETER FOR CAROTENOID PIGMENTS IN FLOUR EXTRACTS OBTAINED WITH WATER-SATURATED *n*-BUTYL ALCOHOL IN THE INSTANCE OF 103 FLOURS

Correlation coefficient between spectrophotometric carotene (ppm. in flour) and apparent density (L) as determined with the Evelyn photoelectric colorimeter	$r = +.997$ (1% point = 0.254 approx.)
Regression equation: Carotene ppm. (water-saturated butanol solvent) = $0.174 + 0.1657\ L$ (apparent density)	Standard error of estimate 0.087 ppm.

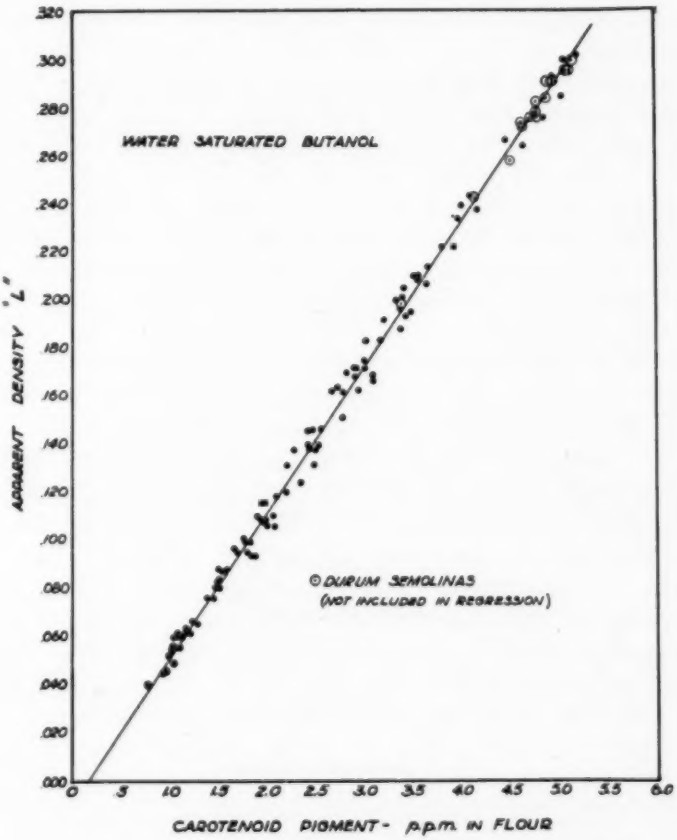

Fig. 3. Statistical data from 103 flours—water-saturated butanol.

a broader spectral region than is the case with the spectrophotometer, it is to be inferred that the butyl-alcohol solvent extracts rather more flavone pigments than the naphtha+alcohol. This conclusion is in opposition to that of Binnington, Sibbitt, and Geddes (1938), who believed that butyl alcohol was more satisfactory in this respect.

Summary

The complex nature of the pigments of wheat and flour is pointed out and certain factors involved in their estimation in flour extracts are considered. The inapplicability of conventional colorimetric procedures to this type of determination is indicated.

Various methods of estimating the pigment content of flour extracts are discussed and the spectrophotometric procedure indicated as most closely approximating the ideal. It is pointed out, however, that it represents a research procedure and that it is not well adapted to routine testing.

The use of photoelectric colorimeters is discussed, their limitations indicated, and the difficulty of standardization pointed out. A particular type of photoelectric instrument, the Evelyn, is briefly described and the agreement secured between two instruments of this manufacture is detailed. This agreement is sufficiently good to permit the use of a single calibration curve with the instruments employed. In the event that these calibration data are to be applied to other instruments of the same manufacture, a check with potassium chromate solutions is suggested.

The results of an extensive series of comparisons between spectrophotometric and photoelectric results are detailed and data presented enabling the construction of calibration tables and curves for both naphtha+alcohol and water-saturated *n*-butyl alcohol solvents. The errors of prediction are 0.048 and 0.087 ppm. respectively for the two solvents. It is pointed out that from a practical standpoint, these errors are of an essentially similar order of magnitude.

Acknowledgments

The authors desired to acknowledge the assistance of Harold Johannson, Ogilvie Flour Mills, Montreal, Canada, who collaborated in the joint testing of the two photoelectric instruments; of R. I. Derby, in securing many of the data presented; and of F. C. Hildebrand for assistance in statistical reduction of the data.

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THE ORIGIN OF THE GAS CELL IN BREAD DOUGH

J. C. Baker and M. D. Mize
Wallace & Tiernan Laboratories, Newark, New Jersey

(Read at the Annual Meeting, May 1940)

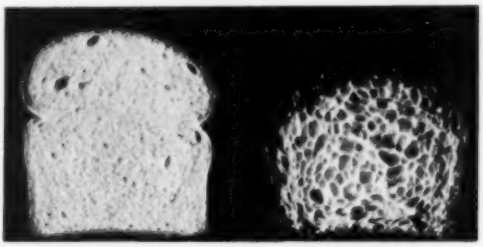
This paper is a study of the conditions and causes that produce gas cells in dough and control their retention. A preliminary survey of bread making would indicate that the cells in dough originate for the following reasons:

1. The gas cells in the endosperm particles are incorporated in the dough.
2. The gas voids between the endosperm particles in the flour are occluded in the dough during mixing.
3. The mixing beats gas into the dough and subdivides it to produce gas cells of small size.
4. The gas pressure caused by yeast will originate new gas cells around the organism.
5. The work applied to the dough after it is mixed, such as folding, punching, rolling, molding and twisting, subdivides gas particles to increase their number.

To test the above hypotheses and to indicate their relative importance, means were designed whereby bread could be produced in which all of the operations of bread making were eliminated as far as possible, thus enabling one variable to be studied at a time. For this purpose "no-time" doughs were employed for the first part of this paper. The molding of doughs or other means which apply work to the dough beyond the mixing operation were eliminated. The dough, immediately after mixing, was gently extruded through a $1\frac{3}{8}$" orifice directly into the pan. This manner of shaping the loaf involves no substantially additional work beyond the mixing process. The loaves were then proofed to standard height and baked.

In order that satisfactory no-time breads can be made, rapid-acting oxidizing agents are necessary, which are capable of producing satisfactory bread with ordinary mixing procedures. For this purpose one can use vanadate catalyst with bromate, as described by MacIntosh (1937), or sodium chlorite can be used according to the process of Hans Pelser (1930). In these experiments sodium chlorite was used. The bread obtained by this no-time process is nearly identical to that obtained by the use of bromate in a two- or three-hour straight-dough process. The main difference is in flavor.

To test the above hypotheses a dough of standard no-time composition was mixed in air with the optimum amount of sodium chlorite and mixing time to produce the best loaf possible from the flour. The same standard baker's patent was used throughout this series of experiments. A similar dough was mixed under identical conditions



Mixed in air Mixed in vacuum

Figure 1

except that the mixing was conducted in vacuum, as described by Baker and Mize (1937). The two no-time loaves are shown in Figure 1.

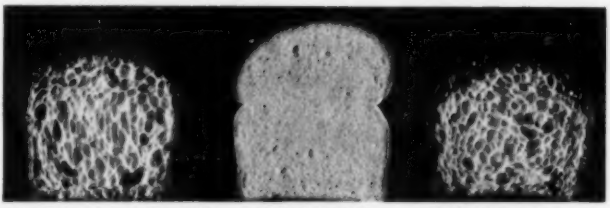
The first loaf, the air-mixed loaf, is normal bread. A similar dough when mixed in vacuum and baked [1] gives the second loaf, which is coarse-textured and thick-walled. The cell walls are entirely devoid of small gas cells, there being no other gas cells in the bread than are visible to the eye in the picture. The walls appear very similar to the beeswax in honeycomb. It is obvious in this bread that either very few cells were present at the end of the vacuum mixing or few were produced by the yeast itself. It is also obvious that the yeast under these conditions is incapable of creating gas cells in a sufficient number to produce ordinary bread. It is very apparent that once a few gas cells are available, the gas produced by the yeast organism diffuses into them and such cells receive the gas of fermentation without creation of further cells. The dough had an expansion during the proofing period equal to that of a dough produced by the ordinary

[1] Baker and Mize (1937) obtained good bread from such vacuum mixes by using 2½-hour straight-dough fermentation methods.

mixing method. One might conclude from this experiment that the yeast organism is incapable of originating gas cells in dough.

The density of the vacuum-mixed dough coming from the mixer was 1.25, and that of the air-mixed dough was 1.15. The difference between these two densities of 0.10 would indicate that the air-mixed dough had about 8% of its volume occupied by gas voids or cells which were produced in the mixing, contrasted to the vacuum dough which obviously will contain no such gas spaces. Possibly the texture obtained in the air-mixed dough occurred because sufficient corresponding small gas voids or cells were produced during the mixing.

Figure 2 shows exactly similar bread with the exception that the first two loaves were mixed in carbon dioxide, of which the first, immediately after extruding into the pan, was placed in a chamber and subjected to air pressure of 80 pounds per square inch for five minutes.



Mixed in CO_2, then subjected to an air pressure of 80 lbs./sq. in. | Mixed in CO_2 | Mixed in vacuum

Figure 2

The third loaf was mixed in vacuum. The three loaves were proofed to standard height and baked.

It is to be noted that the vacuum loaf is similar to the one described above. The carbon dioxide loaf is similar to the air-mixed loaf, with possibly slightly improved texture. The first loaf, which was also mixed in carbon dioxide and then subjected to pressure, appears identical with the loaf mixed in vacuum. It is to be noted that the density of the loaf coming from the pressure chamber was 1.22, whereas going into the chamber it had a density of 1.15. Obviously the pressure has driven the gas contained in the gas cells or occluded in the dough into solution and has produced a solid compact dough similar to that mixed in vacuum.

The only difference observable between the carbon dioxide pressure loaf and the vacuum loaf in this instance was that the pressure loaf proofed in a slightly shorter time, doubtless because of its larger initial supply of carbon dioxide. It is again obvious that the yeast organism is unable to originate gas cells to produce bread texture. It is also obvious that the texture present in the second loaf must have been present in the dough coming out of the mixer. The 8% of gas volume

present in this dough is apparently distributed as extremely small voids or cells which can be considered as gas nuclei, which, upon expanding, as gas feeds into the cells, produce the resulting texture in the bread.

Figure 3 shows the same two loaves of bread mixed in carbon dioxide as Figure 2, subjected to the same treatment of no pressure and pressure. Loaves 6 and 7 of this chart are two exactly similar loaves mixed in air. The pressure which was applied to Loaf 7 was exactly the same as that applied to Loaf 5, but when released gave a dough of a density of 1.17. Very little effect was produced upon the texture of the resulting bread, only coarsening or damaging it to a slight

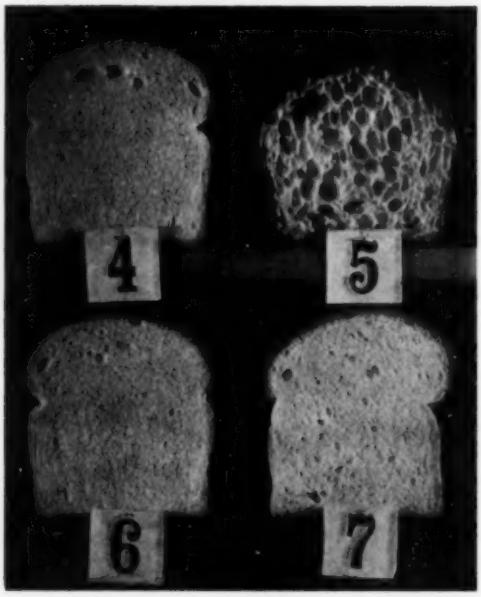
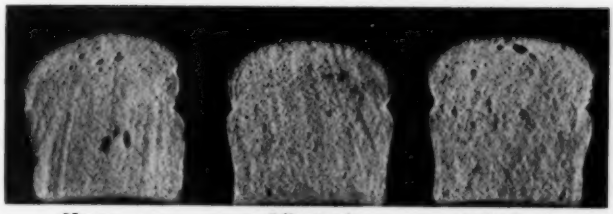


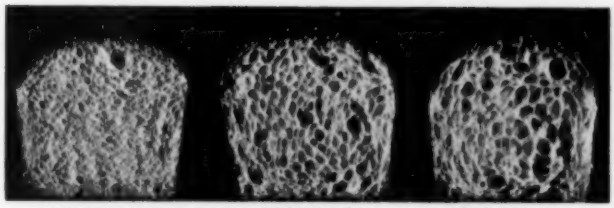
Figure 3

degree. It is obvious that air, being a relatively insoluble gas, was not forced into solution by the pressure and the voids or cells in the freshly mixed dough were thus not eliminated. Hence the dough retained the gas cell nuclei which were produced in the mixing process. Further illustration and proof are shown in the following figures.

Figure 4 shows a series of doughs mixed in carbon dioxide which were subjected to increasing pressures up to 80 pounds per square inch for five minutes, showing clearly that as the pressure increased and approached 80 pounds the number of gas nuclei present rapidly decreased and approached the vanishing point.

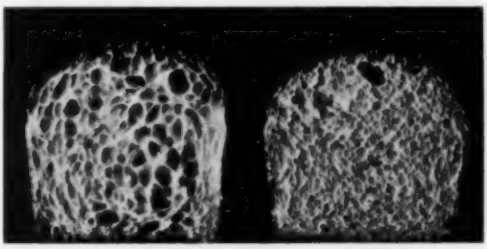


No pressure 5 lbs./sq. in. 10 lbs./sq. in.



20 lbs./sq. in. 40 lbs./sq. in. 80 lbs./sq. in.

Fig. 4. Effect of air pressure applied immediately after panning, to doughs mixed in carbon dioxide.



Before proof One-half proof

Full proof No pressure

Fig. 5. Effect of air pressure applied at 80 lbs./sq. in. at different periods during pan proof on doughs mixed in carbon dioxide.

Figure 5 illustrates the same point in a different manner. A series of carbon-dioxide-mixed doughs were extruded and then subjected to pressure at different times during proofing, from no proof to full proof, 80 pounds pressure being applied for five minutes in each case. As the volume of carbon dioxide in the dough increased with fermentation the pressure was unable to drive it all into solution and many of the

gas nuclei, though doubtless reduced in individual size during the compression, contained too much gas to be entirely dissolved and thus retained their identity.

Figure 6 shows a loaf mixed in vacuum and three other loaves which were mixed part of the time in vacuum and part of the time in air. Loaf 1 was mixed seven minutes in vacuum, Loaf 2 four minutes in vacuum and three minutes in air, Loaf 3 six minutes in vacuum and one minute in air. Loaf 4 was exactly similar to Loaf 3 except that the yeast was added only during the last minute of mixing. It is obvious that the gas cells and nuclei which were eliminated in the

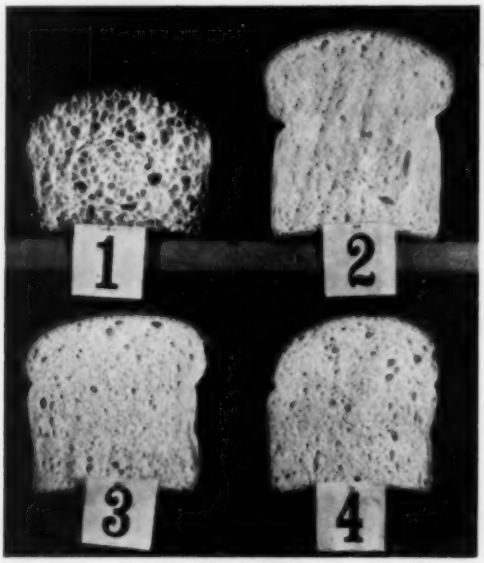


Fig. 6. Functions of the mixing period: (1) vacuum mix 7 min., (2) vacuum mix 4 min., then air mix 3 min., (3) vacuum mix 6 min., then air mix 1 min., (4) vacuum mix 6 min. without yeast—then air mix 1 min. with yeast.

vacuum mixing were returned to the dough almost completely in one minute of mixing in air and were completely returned to the dough in three minutes of mixing in air, producing a loaf equal to or better than the seven-minute optimum for this dough shown in other figures. It is also apparent that the presence or absence of yeast has little effect upon this mixing. It is obvious that one can destroy all gas cells and nuclei in dough which may have originated from gas voids in the endosperm or which may be occluded from gas carried between the flour particles or which were beaten in during the early stages of mixing, and entirely replace or substitute for such gas voids, those produced during a relatively short period of mixing in air.

It now becomes apparent that the purpose of mixing doughs is not primarily that of emulsifying gas. The full seven minutes of mixing time which was required to produce good bread from these doughs is apparently not needed for emulsification purposes. Evidently the mixing must do something else to the dough.

Figure 7 was a study designed to show the effect of mixing time upon oxidized and unoxidized doughs. The left half of the figure with the lower numbers are all unoxidized doughs. The right half, with the higher numbers, are all doughs oxidized to produce an optimum loaf when mixed in air for seven minutes. All doughs were produced in the same manner as before described. The top row of doughs were

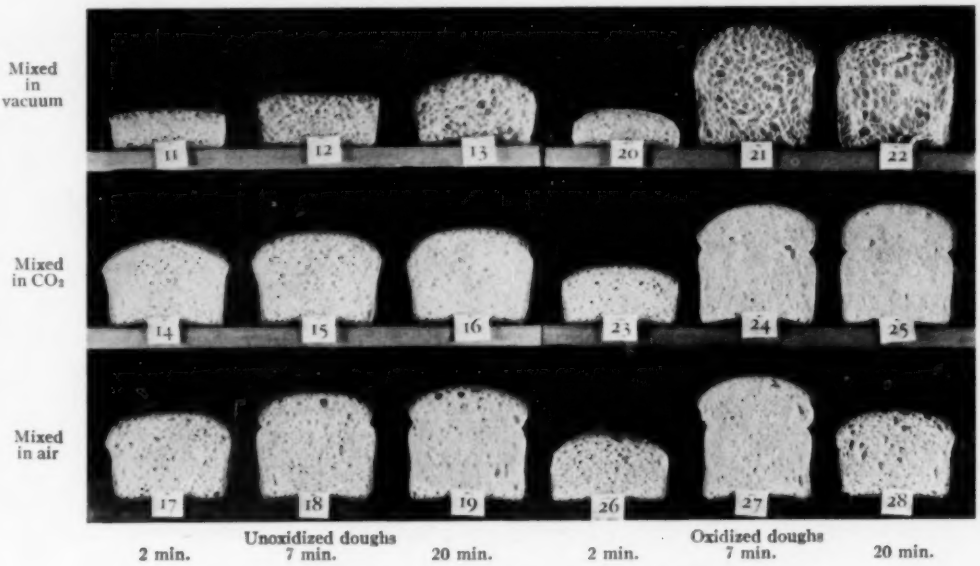


Fig. 7. Effect of mixing time in vacuum, carbon dioxide, or air.

all produced in vacuum with mixing times of two, seven, and twenty minutes in each oxidized and unoxidized series. It is to be noted that with the unoxidized doughs the prolonged vacuum mixing has produced only a slight improvement in its quality. In no case is this unoxidized dough able to retain gas or give evidence of much cell structure in the finished bread. In the oxidized series a remarkable change occurs between the two minutes and seven minutes period of mixing. Though these six vacuum doughs were all of identical density coming from the mixer it is apparent that the short period of mixing has assembled the dough in such a manner that little gas can be retained by the dough during the proofing and baking process, whereas

longer mixing renders the oxidized dough capable of retaining substantially all of the gas generated during proofing and bakes with a normal oven spring. It is also obvious that twenty minutes of mixing has substantially no additional effect upon the dough.

The middle shelf of breads in this figure were all produced by mixing in carbon dioxide; otherwise they were identical with the vacuum loaves immediately above them.

It is apparent that in the unoxidized dough very slight change is produced by altering the mixing time, all of the breads being of poor quality and typical of unoxidized no-time bread. Apparently the short period of mixing in this case has assembled the dough sufficiently to produce nearly as good bread as the long period of mixing. However, in the oxidized doughs a decided and pronounced effect is produced by increasing the length of mixing, a two-minute period being wholly inadequate to produce any appreciable retention of gas in this dough, whereas a seven-minute period has apparently assembled the dough in such a manner that the gases are retained. Longer mixing, up to twenty minutes, apparently has done no damage. It is now apparent that the reason the full seven-minute period is required to make optimum bread from this dough is that it produces an assembly of the dough in such a manner that the gas cells become gas-tight and retain the gases.

The bottom row of bread shows similar doughs mixed in air, the only difference here being that during the air mixing the oxygen has produced an improving effect in the unoxidized series and a possible improving effect for the seven-minute period in the oxidized series, followed by serious damage with further mixing.

In order to determine whether the difference between bread produced from unoxidized doughs as compared to bread produced from oxidized doughs is due to a fundamental difference in the manner in which gases emulsify with a resulting difference in the gas nuclei present in the dough, or whether the difference is caused by coalescence in the first instance and no coalescence in the other, a series of doughs (Figure 8) was prepared with increasing degrees of oxidation and baked by the regular procedure, resulting in the bread shown in the first column. It is to be noted that the increasing oxidation has produced marked improvement in the bread, followed by damage.

The second column shows the dough from which this bread was baked, frozen as it was ready to enter the oven after it had reached full proof. This picture was obtained by proofing the doughs in pup tins and immersing them in a dry-ice and acetone bath, which resulted in almost instantaneous freezing of the outer surface of the dough and complete freezing in a very short period of time. When freezing was

completed, the doughs were removed from the pans and a layer chopped off with a very sharp blade, exposing the cell structure of the rapidly frozen portion of the dough. This shows the condition of the dough at the time it enters the oven. The improvement produced in the bread by oxidation is apparent in the frozen dough.

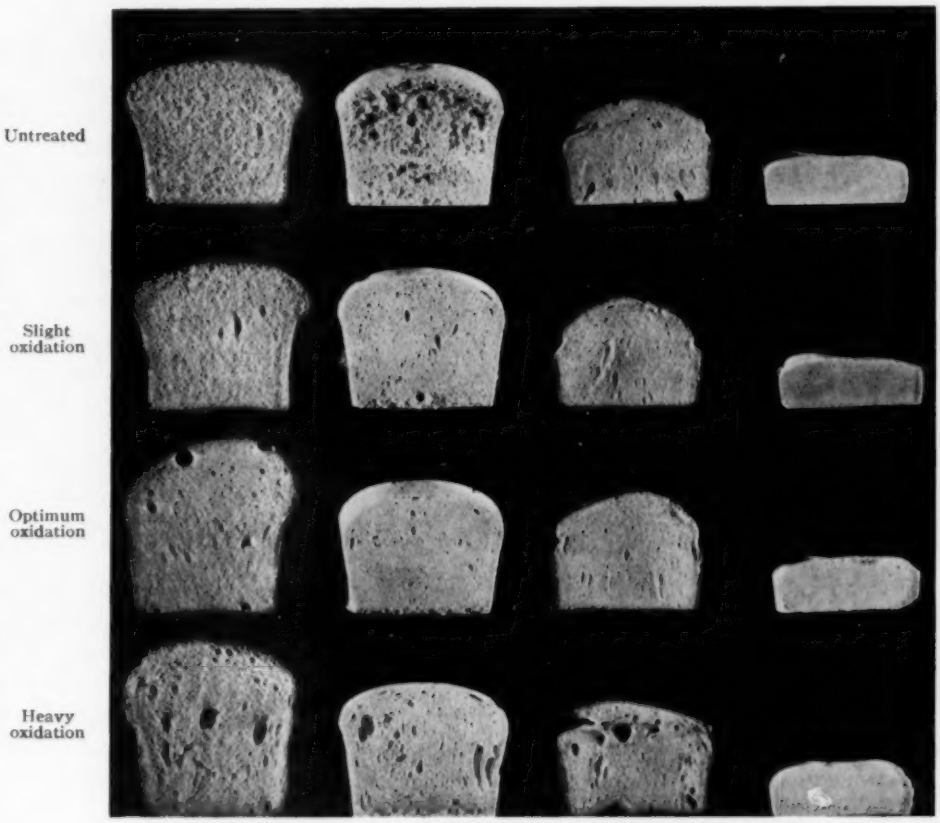


Fig. 8. Effect of oxidation on doughs frozen or baked at ¼ proof and at full proof.

Columns 3 and 4 (Figure 8) contain two similar series of breads and frozen doughs which were baked and frozen respectively after the first quarter of the proofing period had elapsed. It is now apparent that the frozen doughs are all identical and the baked breads are all substantially identical. One can conclude from this information that the number of gas-cell nuclei present in unoxidized dough at the end of the mixing period is substantially the same as the number present in oxidized dough. Also, one can conclude that the differences between

these two types of dough are due to the coalescence of the gas cells which occur during the proofing and baking period. In other words, the difference between these unoxidized and oxidized doughs is that in the former the cells are unable to expand sufficiently to accommodate the gas without breaking and joining together to make larger cells.

It is apparent by comparing the results in Figures 7 and 8 that the unoxidized doughs assemble quicker than do the oxidized doughs but they also coalesce more readily and lose their cell structure.

From the above experiments one can conclude that the gas nuclei in doughs can all be substantially produced by the beating-in action in the mixer and that the other sources of gas cells proposed at the beginning of this paper are either not needed or are without apparent effect upon the cell structure of the dough.

However, hypothesis No. 5—stretching, folding, punching, rolling, molding, and twisting—is one on which no information is shed by these experiments, for as planned, all of these actions upon the dough have been eliminated. It is already apparent that good textured bread can be produced and obtained without their use. The following experiments were performed to shed light upon the effect of these operations upon cell origin in bread making. However, it was not possible to retain the no-time dough technique as the operation of punching requires a period of fermentation. Therefore, subsequent experiments were made upon bread baked with a period of fermentation prior to panning.

Figure 9 shows effects of the molding operation upon the size of bread cells. All of the loaves shown in this chart were produced from oxidized doughs mixed in carbon dioxide in exactly the same manner as described above for no-time doughs. They were then put under 80 pounds pressure for five minutes to destroy all of the gas nuclei produced in the mixing operation. Loaf 1 was then extruded. Loaf 2 was passed through a machine molder immediately after releasing pressure. The subsequent loaves were molded after increasing periods of fermentation—Loaf 3 being molded after 20 minutes of fermentation, Loaf 4 after 40 minutes, Loaf 5 after 60 minutes. Loaf 6 was given a double molding operation, one at the end of 40 minutes and then again 20 minutes later. The loaves, after molding, were panned, proofed, and baked in the regular manner.

It is to be noted that the molder when used immediately after release of the pressure has added no cell structure as shown by Loaves 1 and 2, whereas, when the molder is used after the dough has had a period of fermentation to enable expansion to occur and the cells to grow in size before going to the molder, improved texture is obtained by molding, as shown in Loaf 3. The cell structure of this fermented

pressure dough before going to the molder must have been of very coarse nature, consisting of few bubbles, as shown by Loaf 1.

Loaf 3 shows that the character of cell structure has been modified by the stretching and rolling operation of the molder so that numerous smaller bubbles have been produced in the dough and are present in the bread. Apparently the molding operation had produced effects in the dough so that the number of gas cells was increased.

Loaf 4 shows that a greater increase in new cells was brought about when the dough was molded after having a 40-minute fermentation period.

However, Loaf 5 indicates that more fermentation up to 60 minutes had little further effect.

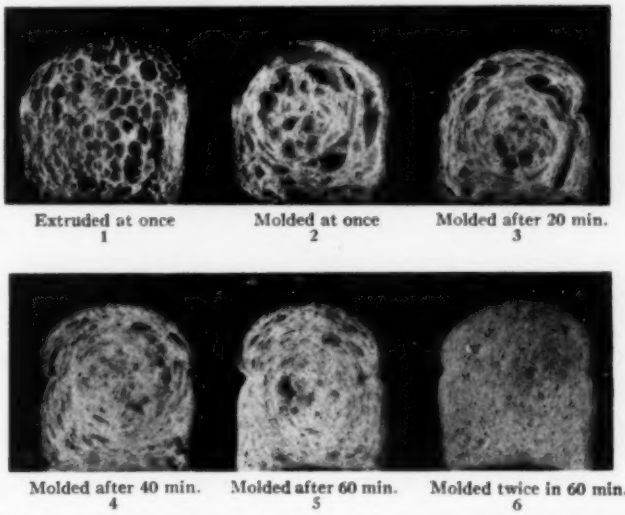
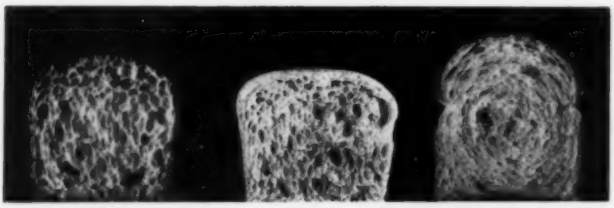


Fig. 9. The effect of molding upon the size of bread cells. Carbon-dioxide-mixed pressure doughs.

Loaf 6 indicates that if a dough which has been given the treatment received by Loaf 4—molding at the end of 40 minutes—is given a second fermentation period of 20 minutes, permitting a second expansion of the dough, and then another passing through the molder, texture is produced in the bread almost equal to that found in a normal loaf of bread. Here, then, is bread appearing normal in its texture characteristic which had substantially no gas nuclei present in it at the time it was removed from the pressure chamber. However, merely by passing such a dough twice through the molder at proper periods of expansion, good texture appears in the bread and a large number of gas cells have been produced in the dough by the act of going through the molder. It is apparent from this experiment

that in a molder the act of stretching and rolling a dough minutely subdivides the larger cells which were produced by the proofing.

Figure 10 pictures three exactly similar carbon-dioxide-mixed pressure doughs that were respectively baked, frozen, or molded at the end of 60 minutes' expansion after mixing. The purpose here is to show that both the baking and freezing of these pressure doughs disclose the same condition of the dough and also to show that the action of the molder has been to subdivide the cell structure of the dough.



Baked Frozen Molded and reproofed

Fig. 10. Cell structure of carbon-dioxide-mixed pressure dough prior to molding and baking.

Figure 11, picturing the relation of oxidation to molding, shows two series of bread. All loaves entered the oven $3\frac{3}{4}$ hours after mixing. In the upper series the proper amount of oxidation was used (5 ppm. of sodium chlorite) to produce optimum molded and punched bread in this period of time. The lower series are identical loaves each handled in exactly the same manner as the corresponding loaf directly above, with the exception that the amount of oxidation (30 ppm. of sodium chlorite) was the optimum required had the no-time technique been used.

The first pair of loaves in each row was mixed in vacuum for seven minutes. The last pair of loaves in each row was mixed in carbon dioxide for seven minutes. The carbon dioxide series and the vacuum series were handled otherwise exactly alike.

Loaves 1 and 4 were extruded directly into the baking pan from the vacuum mixer and allowed to stand in the pan $3\frac{3}{4}$ hours before baking.

Loaves 2 and 5 were identical to Loaves 1 and 4 but were put through a machine molder after $2\frac{1}{2}$ hours of fermentation, proofed $1\frac{1}{4}$ hours and baked.

It is to be noted that with light oxidation, the extruded dough mixed in vacuum coalesced and collapsed during the prolonged proofing and collapsed further during baking, as shown by Loaf 1. The corresponding heavily oxidized vacuum-mixed dough No. 4 in the second row continued to expand in the pan throughout the entire $3\frac{3}{4}$ hours' proofing period and at no time showed evidence of collapse. Neither did this dough collapse when placed in the oven, but gave a slight

additional oven spring. Apparently the heavy oxidation has produced a tough, expansible gluten structure which does not coalesce or collapse like the loaf immediately above. However, when these two doughs were passed through the machine molder at the end of the 2½ hours' fermentation period and then proofed for normal proofing time, they both produced good bread but the loaf having the lesser degree of oxidation, No. 2, produced very much better bread than the strong-structured dough of heavy oxidation, loaf No. 5. Prior to passing through the molder, Loaf 2 was a flat, poor dough with apparently poor gas-retaining properties. However, upon being stretched and molded the resulting loaf shown was obtained. Apparently the action

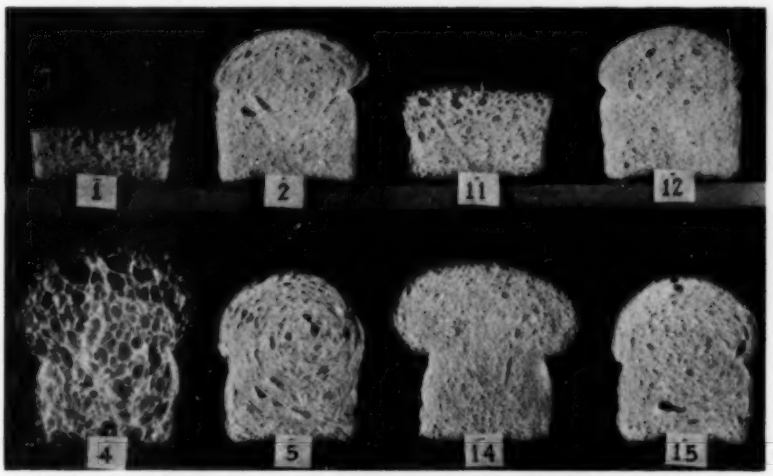


Fig. 11. The relation of oxidation to molding.

of the molder in this case was to produce a greatly increased number of gas cells in the dough, which retained their identity through the normal proofing and baking period. However, in the heavily oxidized series the action of the molder gave no such fine gas-cell structure as was obtained in the case of the low oxidation, though the dough going to the molder possessed a much stronger and more voluminous gas structure. Apparently the molder was unable to produce from this gas structure as many fine cells as it was able to produce from the much softer, stickier, and more easily ruptured dough.

In the carbon-dioxide series of this chart Loaves 11 and 14 show a very similar action to Loaves 1 and 4. The dough with low oxidation was unable to retain the gas and a great amount of coalescence oc-

curred, whereas the heavily oxidized dough mixed in carbon dioxide also had a large volume but differed from the vacuum loaf, No. 4, in having a relatively fine cell structure. When these two doughs were put through the molder, the one with the low oxidation, Loaf 12, produced bread with very fine cell structure and good volume, as in the vacuum series, wheras the one with the high degree of oxidation, Loaf 15, suffered an actual loss in cell content. The molder was able to take the soft, relatively sticky, partially oxidized dough and create a relatively large number of gas cells, whereas it was unable to mold the heavily oxidized dough which still possessed many gas cells without destroying by its violent action a portion of this cell structure. Apparently the reason light oxidation is used with long fermentation is

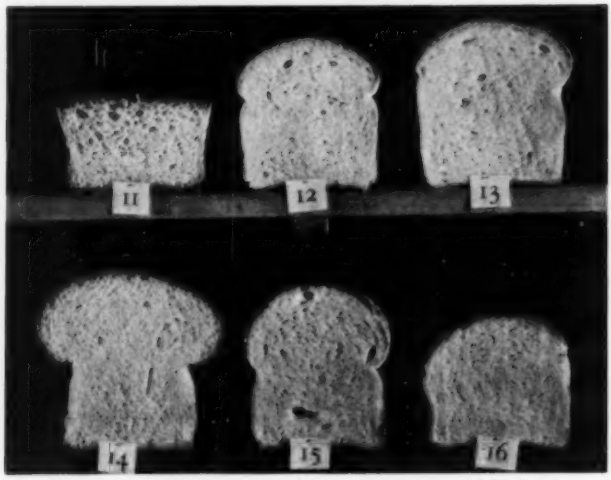


Fig. 12. The relation of oxidation to molding and punching.

because molding is capable of reviving and strengthening the cell structure. Where molding is applied immediately after mixing, prior to fermentation, heavy oxidation needs to be used to obtain desirable results. Molding of heavily oxidized dough after fermentation may cause destruction of the existing cell structure.

In order to study this problem further, Figure 12 contains the four carbon-dioxide-mixed doughs of the immediately previous figure with the additional Loaves 13 and 16, which were produced by punching the doughs twice during the fermentation period. In other respects they were handled identically to Loaves 12 and 15, which were molded only. It is now obvious that the punching operation has further improved the dough with a low degree of oxidation but it has very markedly

damaged the loaf with the high degree of oxidation. It is now apparent that the punching and molding operations of baking have beneficial action upon doughs which are sufficiently soft, sticky, and extensible for the operations to subdivide the existing gas cells. These same operations have a damaging effect upon doughs which are heavily oxidized.

Summary

The manner in which the gas cell in bread dough originates has been studied. Five hypothetical sources of the gas cell have been examined. For this purpose means were devised for making no-time bread of fine texture by using rapid oxidizing agents. An apparatus was built for extruding doughs so that the different sources of the gas cell's origin could be studied separately.

By mixing doughs in vacuum or by subjecting doughs to high pressure, the cells in the dough can be substantially eliminated. Their subsequent development can be used to show which of the sources of the gas cell are important.

It was thus shown that the yeast organism is incapable of originating gas cells in the dough itself. The entrained gases in the endosperm or occluded in the flour or beaten in during an early stage of mixing are of little or no consequence as a source of gas cells, in a properly developed dough.

The latter portion of the mixing period is capable of emulsifying all of the required gas to initiate the resulting gas cells which produce the texture in bread.

Punching and molding do not introduce any new gas cells into bread dough but create a greatly increased number of cells by subdividing those already present.

It is apparent that the texture in bread dough originates from those operations which apply work to the dough.

By quickly freezing dough just ready to go to the oven one can show that all of the cells found in bread exist in the dough when placed in the baking pan. The main differences in bread texture are due to coalescence and breaking of cells during molding, proofing, and baking.

The difference between oxidized and unoxidized doughs is not due to the amount of emulsification of which they are each capable. Rather they differ because an unoxidized or green dough does not have the capacity to prevent coalescence of bubbles during proofing and baking as does an oxidized dough. An overoxidized dough, on the other hand, is incapable of standing the severe action of punching and molding without breaking cells existing in such doughs.

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THE STRUCTURE OF THE GAS CELL IN BREAD DOUGH

J. C. BAKER

Wallace & Tiernan Laboratories, Newark, New Jersey

(Read at the Annual Meeting, May 1940)

Fermenting bread dough, during its active expansion period, consists of a mass of gas bubbles which may be gas-tight. The bubbles have walls of dough that form an apparently gas-tight envelope. The structure and properties of the walls must of necessity determine the character of the dough.

Many studies of dough properties have been made and theories concerning bubble structure advanced. The wall of the dough bubble has been conceived of as a matrix of gluten and starch which, by their mutual adhesive properties, give it strength. Swanson and co-workers propound the theory that the properties of the wall structure are due to a glutinous network of strands. The gluten and starch structure is rendered gas-tight by the capillarity and surface properties of water which lies in and between these materials. In other theories the gluten is characterized as forming a continuous phase of protein material around the starch. The continuity and properties of the gluten phase in this theory determine the bubble's character.

This paper was initiated during a study of the dilution of dough by slowly adding 3% brine [1] during gentle stirring. Subsequent centrifuging of this diluted dough separates the ingredients into distinct layers. It was observed when unoxidized diluted doughs were centrifuged that the ingredients separated into three layers, namely starch at the bottom, gluten in the middle, and a briny layer on top. An oxidized dough,[2] when similarly diluted with 3% brine, developed a fourth layer above the brine. As the oxidation was increased the amount of this layer increased and became an appreciable proportion of the entire depth of the material in the tube.

[1] Sufficient brine was used so that the total liquid added from the beginning was equal to two and one-half times the weight of the flour.
[2] Oxidation was accomplished by adding sodium chlorite up to 100 parts per million of the flour.

This top layer was carefully removed and diluted with more 3% brine. Mixture occurred readily and the material dispersed easily through the brine. When the admixture was made with only gentle stirring, so that the character of the material was retained as far as possible, it was noted that the layer separated into a glutinous foam that floated on the top and starch which settled by gravity to the bottom of the diluting liquid. This top layer could be removed and upon gently mixing in more 3% brine, would again separate to the top, appearing now like thin, translucent protein bubbles. These bubbles, which came from the original dough, were removed and examined for their properties. They were found to contain, on the anhydrous basis, approximately 45% protein. This bubbly material when placed in a vacuum chamber would expand to 10 volumes without breaking of the bubbles and, on release of the vacuum, would return to nearly its original condition. This expansion could be repeated more than once on the foam from the more highly oxidized doughs.

Apparently upon dilution of oxidized dough many of the smaller bubbles retain their integrity throughout the entire process. They are separated from the dough as such, and can be further washed to remove starch. Thus the material of which many dough-bubble walls is composed is shown to be essentially glutinous in character, containing approximately 45% protein and 55% fine starch. Apparently the bubbles which are separated from the dough depend upon a wall structure that is not dough in character.

A further series of observations was made on doughs fermenting in glass expansion jars. These present a bubble structure against the glass wall of the jar in such a manner that the interior surface of the bubbles can be observed. The walls of bubbles from unoxidized doughs are apparently dull and do not reflect light as would a soap bubble. Examination of the character of the bubbles in more highly oxidized doughs presents marked differences from those seen in unoxidized dough. Within the very small bubbles the surfaces are dull and not highly reflective but as the bubbles grow larger the surface becomes shiny and smooth with good reflection.

It is also to be noted that as these doughs reach a high degree of expansion there is apparent a new type of bubble having nearly transparent walls. Apparently some of the glutinous material has been able to free itself sufficiently from the starchy portion of the dough so that bubbles can be formed that are approximately transparent and the walls of which must of necessity be essentially glutinous in nature. Evidently during fermentation there is a tendency of the gluten to draw itself together away from the starch. In those bubbles which present a glossy surface the walls are probably glutinous at the surface

with relatively few of the larger starch granules in the surface to break up their reflective properties. Obviously one cannot collect such bubbles and determine their wall characteristics; neither could they be photographed.

When highly oxidized doughs are proofed to excessive heights and baked, a few of the films which constitute the bubble walls become sufficiently extended so that extremely thin, flat bread films can be collected and mounted. Good photomicrographs of the bread films were thus obtained with all parts sharply focused. The films were placed upon a microscope slide and covered with $n/100$ iodine solution. It was noted that the iodine solution did not color the bread film except along the edges where the film had been cut from the bread and on its surface only in spots where there were holes. The remaining surface of the film would not stain blue, though the iodine solution was removed with a blotter and repeatedly renewed. The bread film swelled in the solution, the aqueous portion of it penetrating through the surface of the bread film but apparently not carrying iodine into the interior, so that starch, if any, in this zone would stain. That starch did exist was definitely proved by scratching the surface of some of this film either before the iodine was applied or while it was in contact. A blue color would appear at the scratch.

It is apparent that these films of bread have walls of impervious glutinous material. That they were glutinous was shown by bathing with picric acid solution, upon which the walls stained a brilliant yellow color.

The accompanying photomicrographs (Figure 1) were taken of the bread film just described. They were taken to include the holes naturally occurring in the film and therefore show the manner in which the walls surrounding the dough bubbles puncture and fail, and thus permit gas to escape.

The upper left picture (No. 1) in Figure 1 shows a film magnified 75 diameters. The fine, wavy material is the glutinous wall stained yellow and the larger round dark objects are the starch granules stained blue. Only the starch surrounding the hole is stained. The picric acid solution has removed the blue from some of the more accessible starch grains. It is to be noted that the wavy material which constitutes the gluten wall of the membrane is continuous. This suggests again a gluten membrane which gives the bubble its gas holding properties. The wavy surface appearance is probably due to shrinkage of the film, after the puncturing of the bubble.

Photomicrograph No. 2 (Fig. 1) is a section of bread film with part of a larger hole, showing similarly a wall of glutinous substance and

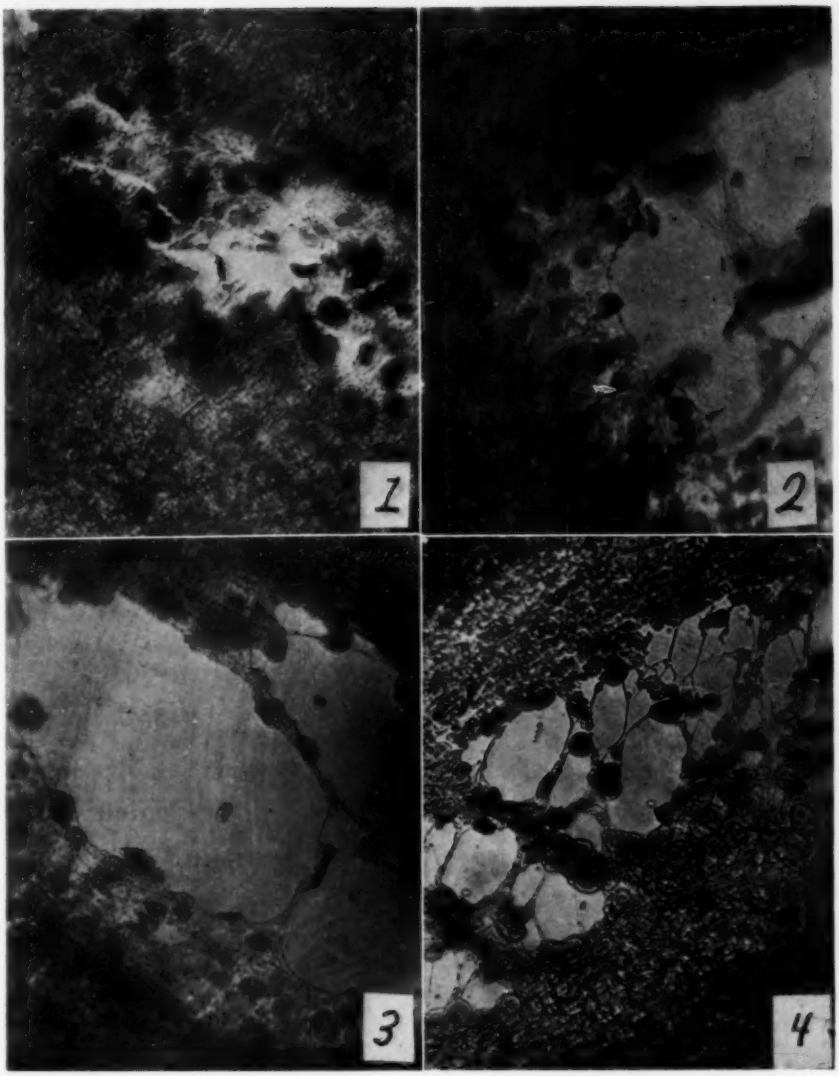

Fig. 1. Photomicrographs of films which form the cell walls in the crumb of overproofed bread. All pictures include ruptures produced in the film by escaping gas. Magnification 75 diameters.

starch particles as they lay in the bread material. This is clearly shown in the center section of the picture.

No. 3 is a similar picture, showing glutinous material pulling away from the film. The fluid character of gluten under the heat of baking becomes evident. It has stringy or fluid properties which permit it to draw out more or less like melted cheese. This is particularly well

shown in No. 4, where the fracture in the wall of the bread membrane is caught in the act of stretching and breaking. The expansion of the loaf must have ceased as the heat coagulated and set the melted proteins as shown. It is to be noted here that as the walls separated they pulled out into gelatinous or fluid strings to which the starch adheres. In many cases it can be noted, in this and the other pictures, that the glutinous material has an identity all its own and that the starch is not of necessity present in the gluten.

Further evidence supporting this theory is to be noted in the character of bread crumb. Bread made from unoxidized or green doughs has a dull appearance with very little sheen and stains easily with iodine, whereas quality bread is notably characterized by what the baker calls "sheen" or a very shiny appearance of the cell walls, and stains more slowly. Apparently unoxidized doughs are characterized by the presence of much starch in the wall, which roughens the surface, absorbs light, and prevents a shiny appearance, whereas in the properly developed doughs gluten is drawn into the surface of the cell wall and gives it a shiny appearance.

One can conclude from this work that the surface of a dough bubble may be either doughy in character, as in an unoxidized or green dough, or it may have a glutinous character, as in oxidized or developed dough.

These experiments suggest those properties which enable a gas bubble to retain its integrity while expanding. In oxidized or developed dough the gluten material has sufficient integrity or continuity so that it will not be torn apart but rather will draw more glutinous material into the expanding surface to supply the needs of the bubble and produce a gas-tight film on the inner surface of the bubble. The dough forms a matrix of glutinous and starch material from which the wall draws the required amount of gluten into its expanding surface by the strength of its elastic properties. In case the gluten does not have sufficient strength to accomplish this result it breaks and expansion of the bubble no longer continues. The properties of the bubble are largely determined by those properties of the gluten which enable it to retain its integrity as a film at the inner bubble surface. If the gluten itself has a relatively low viscosity and hence is somewhat fluid in character, it will break rather than pull more of the glutinous material into the film. Yet, in order to flow from the matrix the gluten must still be somewhat liquid in nature, but also tenacious. This tenacity is obtained because its viscosity is relatively high in comparison to its modulus of elasticity, as propounded by Halton and Scott Blair (1937), whereas the flow may be obtained because the numerical values of the viscosity and the elasticity are low.

A further condition in the dough that may affect the travel of gluten from the matrix into the surface film is affinity or adhesion between the starch granules and the flowing glutinous material. This affinity must be very much affected by the character of the starch granule surface. Thus may be explained the observation recently reported by Sandstedt, Jolitz, and Blish (1939), who describe the making of bread by recombining gluten and starch. They found that when the starch was treated with hypochlorite or chlorine the resulting bread was of very inferior quality to that which they made with unchlorinated starch. They also found that when they treated this chlorinated starch with α-amylase for a sufficient period of time the bread-making properties of the reconstituted starch-gluten dough were recovered. It is probable that an effect of chlorine, and later of α-amylase, is on the character of the surface. In view of the theory just expounded it is very possible that such alteration of the starch surface by causing a change in resistance to gluten flow may have a profound effect in determining the bread-making properties of dough.

Application of the Theory

It is evident from the experiments given that the wall of the gas bubbles in developed dough is essentially glutinous in character and that during the expansion of a bubble, gluten must necessarily be drawn to the surface by the expansion to supply the necessary wall to retain gases. It is also apparent that when a bubble which has a glutinous wall collapses, the surface material is drawn together and will produce a glutinous nucleus in the dough mass. It is then possible that the beneficial effect obtained upon dough by fermentation and the subsequent punching is due to the multiplying of such glutinous nuclei within the dough, providing the material for better bubble structure on subsequent expansion.

Speculations can be made concerning the mixing necessary to obtain dough development. The kind of mixing applied to doughs or used in forming them is important. Devices which rub, cut, or tear, damage rather than improve dough. They can only be used where their damage is later corrected during fermentation by punching or molding operations, or by subsequent proper mixing technique. Desirable mixing methods involve operations that stretch, fold, or squeeze the dough, such as are obtained in the Swanson or in the McDuffee mixer.

A dough-mixing machine was built which operated by rubbing dough between a revolving drum within a cylinder. A second device operated like a mixing conveyor, using many pins on both shaft and wall, and so operated that the chamber was kept filled with dough.

A third device was a small commercial laboratory batch dough mixer with a cover. When operated full of dough no stretching or folding took place. All of the above devices were incapable of mixing and developing dough so that even passable bread could be made under any condition of oxidation, speed, time of mixing, absorption, etc. There appears to be some magic in the stretching of dough which cannot be obtained otherwise.

It is well known that flour must be granular in order that good bread can be made. It is also well known in the industry that all mixing operations must be of the nature of stretching. In view of the conclusion here that a bubble requires a glutinous wall to function well, it would appear that the combination of granular endosperm particles plus stretching operations during the mixing may result in drawing glutinous material from the endosperm matrix and concentrate it between the surfaces of the endosperm particles. Such a result would provide ready-made material for gaseous nuclei with glutinous cell walls. The emulsed bubbles which are beaten into the dough can thus start to expand with initially formed glutinous walls.

With flour ground to a powder so that all the endosperm particles are disintegrated, such a phenomenon as classification of the gluten by mixing would appear difficult to accomplish. It is also apparent that overmixing of a dough would disintegrate the endosperm particles so that a result somewhat similar to overgrinding of flour might be obtained. Classification of the dough ingredients by further mixing would be rendered unlikely. However, fine grinding or overmixing, though making classification by mixing unlikely, would not preclude classification of gluten by bubble expansion and subsequent collapse of the bubble in punching, thus explaining some of the beneficial effects and corrective action obtained upon doughs by fermentation.

Summary

The washing out of bubbles from oxidized or developed bread doughs by careful dilution and rinsing methods has shown that these cells possess essentially thin glutinous walls which are capable of expansion in a similar manner to bread dough. Examination of the cell surfaces of a similar fermenting dough in glass jars shows the development of shiny surfaces. Later some of the bubbles develop transparent gluten walls. Similar observations of baked bread from oxidized or developed doughs shows them to possess shiny surfaces on the interior of the cell walls in contrast to the undeveloped doughs which possess bubbles of dull surface.

From greatly expanded bread doughs, thin, flat sections of bread films were obtained, stained, and photographed. By the manner in

which the stains were taken and the character of the ruptures shown in the film which were produced during the baking, one could note that they were covered with an impervious film of gluten on each surface and that starch was carried between the surfaces and could be stained by iodine only through points of rupture or damage.

These series of observations indicate that in a developed dough (particularly by means of oxidation) the bubbles acquire a thin film of gluten lining the surface which gives the bubble its shiny appearance and must contribute greatly to its gas-tight properties and strength. It is conceived that this film is drawn to the surface because the gas nucleus from which the bubble originated started in a glutinous core. As the bubble was expanding the required amount of gluten to satisfy its surface needs was drawn from the starch-gluten matrix of the endosperm material. The properties that enabled this to occur may be controlled by the viscosity and fluidity of the gluten and by the amount of adhesion of the gluten to starch.

These experiments suggest that the beneficial effects obtained from punching doughs is due to the collapsing of such glutinous cell surfaces during the punching, thereby providing glutinous centers in which the contained gaseous nuclei can develop with subsequent tighter gluten walls.

It has been shown that the mixing develops dough by means of stretching and folding which occur during the mixing operation. It is conceived that the stretching operation thereby draws gluten from the endosperm particle matrix to the surface of the particle and produces there gluten concentrate, which is available for holding gas nuclei produced in the mixing, thus partially explaining the development which is acquired during the mixing period.

Acknowledgment

Much help was received in performing the experiments described in this paper from Dr. H. K. Parker, Mr. M. D. Mize, Mr. Harry M. Phillips and Miss Bessie Marks.

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THE EFFECT OF WHEAT TYPE, PROTEIN CONTENT, AND MALTING CONDITIONS ON THE PROPERTIES OF MALTED WHEAT FLOUR[1]

W. F. GEDDES,[2] F. C. HILDEBRAND,[3] and J. A. ANDERSON[4]

(Read at the Annual Meeting, May 1940)

In recent years the production of malted wheat flour for use in increasing amylase activity of flour deficient in this respect has assumed wide commercial importance. There is, however, little published information on the effects of variety, environment, and malting conditions on the properties of wheat malt, although many investigations have been made of the effects of these factors on barley malt.

Early work on the germination of wheat, which has been reviewed by Bailey (1925), was largely confined to studies of the effect of germination time. In general, these studies revealed that the extent of germination must be carefully controlled in order to avoid undesirable effects. In later investigations Sherwood and Bailey (1926a, 1926b) confirmed and extended these observations and suggested that germination periods longer than three days were unsuitable, probably because of excessive proteolytic activity in malts produced under these conditions. The importance of short germination and minimum development of proteolytic enzymes is also stressed in a patent issued to Schreier (1934).

Since the publication of these papers, experimental malting equipment has been developed, primarily for use with barley, which permits of precise control and investigation of steeping, germination, and kilning conditions. With such equipment available, it seemed desirable to reinvestigate the effect of germination time on the properties of malted wheat and to study the importance of additional factors. Accordingly the present study was designed to obtain preliminary information on the effect of type of wheat, protein content, steeping level, germination time, and kilning temperature on the value of the malt for diastating purposes. In order that clear-cut results might be obtained with a relatively small investigation, type was represented by hard red spring and durum wheats and each of the other variables was studied at two widely different levels. A factorial design was thus obtained in which samples

[1] Paper No. 1817, Scientific Journal Series, Minnesota Agricultural Experiment Station; No. 18, Journal Series, General Mills, Inc., Research Laboratories; No. 180 of the Associate Committee on Grain Research (Canada); and No. 915, National Research Council of Canada.

[2] Formerly Chief Chemist, Grain Research Laboratory, Board of Grain Commissioners for Canada; now Professor of Agricultural Biochemistry, University of Minnesota, St. Paul, Minnesota.

[3] Research Chemist, General Mills, Inc., Research Laboratories, Minneapolis, Minnesota.

[4] Formerly Biochemist, National Research Laboratories, Ottawa, Canada; now Chief Chemist, Grain Research Laboratory, Board of Grain Commissioners, Winnipeg, Canada.

of high- and low-protein hard red spring and durum wheats were malted by each of eight methods. The 32 malts were milled, and the amylase and protease activities of the resulting flours and the effects on baking quality of adding them to a low-diastatic flour, were studied.

Experimental

Materials.—Two samples of Western Canadian hard red spring wheat of 14.3% and 12.1% protein content and two samples of Western Canadian amber durum wheat of 14.2% and 12.5% protein were employed. These were obtained by compositing residual lots of No. 1 Northern and No. 1 Canada Western grades from the 1937 protein survey collections of the Dominion Grain Research Laboratory. The range in protein content of the subsamples in each series did not exceed 1%. Thus it will be seen that the samples comprised two widely different types of wheat at two protein levels differing by approximately 2% and were fully representative of wheats available in commercial quantities.

Malting.—Eight malts were made from each sample of wheat using eight different treatments, representing the combination of steeping to 44% and 40% moisture content, germinating for 5 and 3 days, and kilning for 12 hours at 100° F. and for 12 hours with the temperature rising steadily from 100° to 130° F. The eight treatments may be conveniently designated by numbers of three digits, the first representing the steeping procedure, the second the germination procedure, and the third the kilning procedure, as shown in the first four columns of Table I.

The available germination equipment consisted of two chambers (Anderson, 1937), each accommodating eight samples. It was therefore necessary to make two batches, each comprising 16 malts. Possible differences between the malts made in different batches were offset in part by confounding the triple interaction between steeping, germination, and kilning with the batch error. Thus treatments 112, 211, 121, and 222 were used in the first batch, and the remaining treatments were used in the second batch.

The malts which were to be germinated for three days were started two days after starting the malts which were to be germinated for five days. In consequence all malts in one batch were ready for kilning at the same time. Two kilns, each holding eight samples, were available, and one was used for each kilning treatment.

Aliquot parts of the wheat samples, representing 350 g. of dry matter, were steeped in quart jars immersed in a water bath controlled at 50° F. At the end of each 11 hours the samples were aerated by

draining off the water and allowing the closed jars to float in the water bath for one hour, after which the jars were filled with fresh water. The times required for each sample to attain a moisture content of 44% and 40% were determined by pilot experiments and proved to be 48 and 24 hours for the high-protein samples and 56 and 30 hours for the low-protein samples.

The wheats were germinated in the equipment described by Anderson (1937) in cylindrical galvanized iron cans (6 × 6 inches) containing 200 ⅛-inch holes. The temperature of the germination chambers was maintained at 54° F. The malts generate considerable heat and their temperature is thus raised 3° to 5° F. above that of the chamber. The green malts were dried in the kiln described in the same paper, using cylindrical 8-mesh wire cages, and the temperatures noted above.

Immediately after kilning the malt was kneaded in a small bag. The sprouts which were rubbed off during this process were separated by sifting and weighed to the nearest 0.1 g. The polished malt was weighed to the nearest 0.5 g. and its dry-matter content subsequently determined in the vacuum oven. Malt yield and sprouts are reported as grams per 100 g. of wheat dry matter.

Owing to a combination of circumstances beyond the control of the authors a period of 18 months elapsed between the making of the malts and their subsequent use. During this period they were kept in air-tight tins at approximately 2° C. While the enzymic activity of these samples may have decreased as a result of such storage, nevertheless it is felt that such changes should not materially affect the validity of comparisons between the different malts. It would normally be expected that storage would tend to decrease absolute differences between samples, but since the variables investigated were studied at widely separated levels, any appreciable effect of any single factor should be apparent.

Milling.—The milling was carried out in a micro mill similar to that described by Geddes and Frisell (1935). The malts were tempered to 12.5% moisture content and allowed to stand 24 hours. With durum malts sufficient water to raise the moisture content to 13.5% was added one hour before milling. In the instance of spring wheat malts this addition was made 30 minutes prior to milling. Samples were milled to a constant yield of 50% malt flour passing a 10XX silk.

As an index of the replicability of the milling technique, nine aliquot portions of a bulk lot of malted wheat were ground by the above procedure and ash and amylase activity of each malt flour were determined. The latter property was evaluated by adding each malt flour to a common base flour at a level of 0.25% and determining the diastatic activity by the Blish and Sandstedt (1933) method as modified by Sandstedt (1937). The respective mean values were 0.363% and 207.0 mg. mal-

tose per 10 g.; in both cases the variation between malts was not significantly greater than the corresponding variation between duplicates. Accordingly, it was assumed that variability in milling technique would not constitute an appreciable source of error.

Analytical methods.—The moisture, ash, and protein content of the experimental material were determined by standard A. A. C. C. methods, and proteolytic activity by the method of Ayre and Anderson (1939). It has been shown by Hildebrand (1940) that this procedure gives results with malted wheat flours which are highly correlated with those obtained by the Landis and Frey (1938) rate-of-gelation method. It has been assumed that both methods afford an estimation of proteinase activity and are little affected by the presence of other types of proteolytic enzymes. These methods therefore should yield results which are of more significance in relation to dough protein structure than procedures which also measure dipeptidase and polypeptidase action.

Amylase activity was estimated by the technique described by Davis and Tremain (1938), as modified by Hildebrand and Geddes (1940). In brief, this procedure is based upon the addition of increments of malt flour to a common base flour, determination of the gassing power and/or diastatic activity of the resulting blends, and calculation of the amount of malt necessary to produce an arbitrarily selected level of either measure. Amylase activity was conveniently expressed as the reciprocal of the dosage thus calculated. In this study, such amylase values were obtained from both gassing-power and diastatic-activity measurements.

Baking procedure.—All flours were baked by a modification of the A. A. C. C. basic procedure, using 5% sugar, mixing for 2 minutes in the Hobart-Swanson mixer, and fermenting for 4 hours. Doughs were punched and moulded with the aid of a National dough sheeter.

Growth and Yield of Malt

If different malting equipment were used it might well prove difficult to obtain malts closely similar to those used in the present study merely by following the malting procedures previously described. In these circumstances it seems advisable to describe the malts not only in terms of the conditions under which they were made but also in terms of growth and yield. The necessary data are summarized in Table I as means for each treatment over all samples.

The different treatments produced malts representing a fairly wide range in growth of roots and acrospires and in yield of roots and dressed malt. Minimum growth was obtained with treatment 222 in which only one root with a length equal to 0.8 of the kernel was formed; the acrospire was only just showing and the yield of malt was 98% of the

TABLE I

AVERAGE EFFECT OF TREATMENTS ON GROWTH OF ROOTS AND ACROSPIRE, AND ON MALT YIELD

No.	Malting treatment			Length compared to kernel length		Moisture content of malt	Yield (dry basis)	
	Steep.	Germ.	Kiln.	First root	Acrospire		Roots	Dressed malt
	Moisture %	*Days*	°*F.*			%	%	%
111	44	5	100	2.0	0.7	10.2	3.8	93.4
112	44	5	100–130	1.8	0.7	8.1	3.8	93.0
211	40	5	100	1.4	0.5	9.9	1.8	96.3
212	40	5	100–130	1.6	0.5	8.2	1.6	96.3
121	44	3	100	1.1	0.4	10.1	1.7	96.6
122	44	3	100–130	1.4	0.4	8.3	1.7	96.3
221	40	3	100	1.0	Just showing	9.8	0.7	98.1
222	40	3	100–130	0.8	Just showing	8.1	0.7	98.0

weight of the original wheat. Maximum growth was obtained with treatment 112 in which three roots were formed, the longest being 1.8 times the length of the kernel; the acrospire grew to a length equal to 0.7 times that of the kernel and the yield of malt was 93%. The remaining treatments produced intermediate results.

TABLE II

MEAN DATA ON GROWTH AND YIELD OF MALT FOR EACH WHEAT SAMPLE

Sample	Protein content of wheat (13.5% m.b.)	Length compared to kernel length		Moisture content of malt	Yield (dry basis)	
		First root	Acrospire		Roots	Dressed malt
	%			%	%	%
Hard red spring	14.3	1.4	0.4	9.1	2.0	96.1
Hard red spring	12.1	1.4	0.4	9.2	1.9	96.1
Amber durum	14.2	1.4	0.4	9.0	2.1	95.9
Amber durum	12.5	1.4	0.4	9.1	2.0	95.9

The mean data for each wheat sample, over all treatments, are given in Table II. Inspection of the data will show that, on the average, all four samples gave almost identical results.

Table III shows the average effect, over all samples, of changing the steeping, germination, and kilning treatment. Increasing the steeping and germination time had the expected results: growth rate and yield of roots tended to increase and yield of malt was decreased. Increasing

TABLE III

AVERAGE EFFECT OF CHANGES IN STEEPING, GERMINATION, AND KILNING TREATMENTS ON GROWTH AND MALT YIELD

Effect of increasing:	Length compared to kernel length		Moisture content of malt	Yield	
	First root	Acrospire		Roots	Dressed malt
			%	%	%
Steeping level	0.4	0	0.2	1.6	−2.4
Germination time	0.6	0.4	0	1.4	−2.4
Kilning temperature	0	0	−1.8	0	−0.2

the kilning temperature reduced the moisture content of the malt and reduced the yield of malt slightly. This last result suggests that respiration continued during the earlier stages of kilning and was more rapid at the higher temperature.

Protein and Ash Content of Malted Wheats and Flours

The average effect of malting conditions on protein and ash content is shown in Table IV and comparable data for the different wheat samples are reported in Table V. In order to facilitate comparisons, the

TABLE IV

AVERAGE EFFECT OF MALTING TREATMENTS ON PROTEIN AND ASH CONTENT OF MALTED WHEAT AND MALT FLOUR [1]

No.	Malting treatment			Wheat		Flour	
	Steep.	Germ.	Kiln.	Protein	Ash	Protein	Ash
	Moisture, %	*Days*	*°F.*	%	%	%	%
111	44	5	100	13.25	1.530	10.75	0.445
112	44	5	100–130	13.22	1.542	10.72	0.440
211	40	5	100	13.35	1.565	11.02	0.452
212	40	5	100–130	13.48	1.560	11.00	0.440
121	44	3	100	13.48	1.558	11.15	0.478
122	44	3	100–130	13.48	1.522	11.18	0.440
221	40	3	100	13.45	1.648	11.32	0.452
222	40	3	100–130	13.62	1.602	11.42	0.450

[1] Protein and ash results are expressed on a 13.5% moisture basis.

data of Table IV are summarized in Table VI, in which only the statistically significant differences are given. The comparisons in Table VI indicate that conditions which tended to increase growth lowered protein content. This is a natural effect of increased germinative activity

TABLE V

MEAN DATA ON PROTEIN AND ASH CONTENT FOR EACH WHEAT SAMPLE [1]

Sample	Original wheat	Malted wheat		Malted flour	
	Protein	Protein	Ash	Protein	Ash
	%	%	%	%	%
Hard red spring	14.3	14.38	1.320	11.89	0.332
Hard red spring	12.1	12.08	1.668	9.51	0.388
Amber durum	14.2	14.24	1.664	12.41	0.604
Amber durum	12.8	12.98	1.612	10.48	0.475

[1] Protein and ash results are expressed on a 13.5% moisture basis.

TABLE VI

AVERAGE EFFECT OF CHANGES IN STEEPING, GERMINATION, AND KILNING TREATMENTS ON MALTED WHEAT AND FLOUR PROTEIN AND ASH

Effect of increasing:	Wheat		Flour	
	Protein	Ash	Protein	Ash
	%	%	%	%
Steeping level	−0.12	−0.056	−0.24	——
Germination time	−0.19	——	−0.39	——
Kilning temperature	——	——	——	——

resulting in greater translocation of nitrogenous materials to the developing roots and acrospires, which were largely removed in dressing the malt. Presumably the decreases registered would be greater if it were not that increased translocation is accompanied by increased respiratory activity. The latter process takes place at the expense of carbohydrates and therefore would tend to increase the percentage of other constituents. The decrease in protein content is greater in the instance of the flours than of the wheats, since translocational activity originates in the endosperm.

In general, decreases in ash content as a result of translocation were too small to be significant.

Amylase Activity

In the measurement of amylase activity, the malted wheat flours were added to a low-diastatic (165 mg. maltose per 10 g. flour) Southwestern patent flour in increments of 0.25, 0.50, 0.75, and 1.0%. In the instance of twelve of the malts, which were found to be low in activity, a 2.5% addition was also made. Diastatic activity was determined in duplicate on this series of diastated flours by the Blish and Sandstedt (1933) method as modified by Sandstedt (1937). Gassing power was

determined as outlined by Sherwood, Hildebrand, and McClellan (1940), the total gas production at 30° C. for five hours being recorded.

Arbitrary levels of 280 mg. maltose per 10 g. of flour and the corresponding level of 204.7 ml. gas production were selected and the amounts of malted wheat flour required to attain these levels were computed as described by Hildebrand and Geddes (1940). The relative amylase activities of the malted wheat flours were expressed as the reciprocals of these values in order that the resulting figures would bear a direct rather than an inverse relation to activity. A statistical analysis of the values thus computed showed that there was no significant difference in amylase-activity values obtained from diastatic activity and gassing power determinations. Accordingly, the mean values for both methods combined were taken as the best estimate of activity for the purposes of this investigation.

The mean effects of the malting treatments over all samples are recorded in Table VII, and for each sample over all treatments in Table VIII. The average effects of all variables on amylase activity which were found to be statistically significant are shown in Table IX. The effect of any one variable, however, was in some cases conditioned by the

TABLE VII

AVERAGE EFFECT OF MALTING TREATMENTS ON AMYLASE AND PROTEASE ACTIVITY OF MALTED WHEAT FLOURS

No.	Steep moisture	Germination	Kilning temp.	Amylase activity	Protease activity
	%	*Days*	*°F.*	*Units*	*Units*
111	44	5	100	3.78	201.0
112	44	5	100–130	3.80	201.8
211	40	5	100	2.70	111.8
212	40	5	100–130	2.87	104.4
121	44	3	100	1.06	106.0
122	44	3	100–130	0.89	104.8
221	40	3	100	0.73	80.0
222	40	3	100–130	0.70	75.6

TABLE VIII

MEAN AMYLASE AND PROTEASE ACTIVITY FOR EACH WHEAT SAMPLE

Sample	Original wheat protein content	Amylase activity	Protease activity
	%	*Units*	*Units*
Hard red spring	14.3	2.37	127.7
Hard red spring	12.1	2.07	84.5
Amber durum	14.2	1.88	154.2
Amber durum	12.8	1.94	126.2

TABLE IX

AVERAGE EFFECT OF WHEAT TYPE, PROTEIN CONTENT, AND MALTING CONDITIONS ON AMYLASE AND PROTEASE ACTIVITY

Effect of:	Amylase activity	Protease activity
	Units	*Units*
Wheat type (hard red spring minus durum)	0.31	−34.1
Increasing protein content	—	35.5
Increasing steeping level	0.64	60.5
Increasing germination time	2.44	63.1
Increasing kilning temperature	—	—

level of one or more of the other variables involved. Thus for example the effect of protein content depended upon the type of wheat and upon the steeping conditions. Where such significant interactions were found, the pertinent means are given in Table X.

The mean value for both protein levels and all malting treatments for malt flours produced from hard red spring wheat was higher than the corresponding value for durum malt flours. However, the difference between these wheat types varied with both protein level and malting conditions. Thus the difference was significantly greater at the higher protein level, with the shorter steeping, and at the five-day germination.

The effect of variation in protein content was not significant, considering both types of wheat over all malting conditions. Differences due to protein were found but they were not consistent. Thus with hard red spring wheat malts the high-protein samples had the higher activity, while with the durum malts the difference was much smaller and in the reverse order. The same sort of effect was observed with respect to the interaction of protein content and steeping time.

Considering now the influence of malting conditions on amylase activity, the greatest effect was that of germination time; in fact this single variable was responsible for over 80% of the total variability encountered. The malts grown for five days showed markedly higher activity for both wheat types at both protein levels and under all conditions of steeping and kilning. There were, however, as shown in Table X, two significant interactions involving germination time, namely those with wheat type and with steeping level. For the hard red spring malts the difference between the five- and three-day germinations was greater than the comparable difference for the durum malts, although this differential response was small compared to the total effect of germination time.

The interaction between steeping and germination represents a somewhat different situation from the interactions previously discussed. As in all interactions, the absolute effect of one factor is conditioned by

TABLE X

SIGNIFICANT INTERACTIONS—AMYLASE AND PROTEASE ACTIVITY

Amylase Activity

WHEAT TYPE AND:

	Protein		Steeping		Germination	
	High	Low	44%	40%	5 days	3 days
Hard red spring	2.37	2.07	2.43	2.01	3.69	0.76
Amber durum	1.88	1.94	2.33	1.49	2.89	0.93
Difference	0.49	0.13	0.10	0.52	0.80	−0.17

PROTEIN AND:

	Wheat type		Steeping	
	Spring	Durum	44%	40%
High protein	2.37	1.88	2.53	1.71
Low protein	2.07	1.94	2.23	1.78
Difference	0.30	−0.06	0.30	−0.07

GERMINATION AND:

	Wheat type		Steeping	
	Spring	Durum	44%	40%
Five days	3.69	2.89	3.79	2.78
Three days	0.76	0.93	0.97	0.71
Difference	2.93	1.96	2.82	2.07

STEEPING AND:

	Wheat type		Protein		Germination	
	Spring	Durum	High	Low	5 days	3 days
Steeping to 44%	2.43	2.33	2.53	2.23	3.79	0.97
Steeping to 40%	2.01	1.49	1.71	1.78	2.78	0.71
Difference	0.42	0.84	0.82	0.45	1.01	0.26

Protease Activity

PROTEIN AND:

	Steeping		
	to 44%	to 40%	Difference
High protein	180.3	101.6	78.7
Low protein	126.5	84.3	42.2
Difference	53.8	17.3	—

STEEPING AND:

	Germination		
	5 days	3 days	Difference
Steeping to 44%	201.4	105.4	96.0
Steeping to 40%	108.1	77.8	30.3
Difference	93.3	27.6	—

the level of the other. In this instance, however, the ratios between activities for the long and short germination times are virtually identical for both steeping levels. In both cases, decreasing germination from five to three days reduced the activity to 25.6% of its original value. In like manner reducing steeping level brought about a lowering of activity to 73.3% of the original value under both germination conditions. In other words, the activity for three-days germination at 40% steeping level was exactly equal to the value calculated from the combined application of the separate effects of germination and steeping. Thus $3.79 \times 0.733 \times 0.256 = 0.71$. It is therefore clear that this interaction in no sense represents an anomalous effect.

With the exception of germination time, the effect of steeping treatment was of the most significance, the higher steeping producing the greater amylase activity. This difference existed for both wheats, at both protein levels, and for the different germination and kilning treatments, but its magnitude was not consistent throughout. As may be seen from Table X, the effect of steeping was greater in the instance of durum wheat, at the high-protein levels and for the longer germination time. It has already been shown that the interaction between steeping level and germination time agrees with the calculated effects of the simultanous application of both factors.

Variation in kilning treatment within the limits tested was without influence on the amylase activity of the malts.

Protease Activity

The protease activity values are also recorded in Tables VII to IX and the significant interactions in Table X. The durum wheat malt flours were consistently and markedly higher in proteolytic activity than the spring wheat malt flours; with regard to the effect of protein, the high-protein samples gave the higher average protease activity but the difference was not entirely consistent. Thus with wheats steeped to 44% moisture, the high-protein malt flours gave protease values averaging 54 units higher than the low-protein malts, whereas in the instance of wheats steeped to 40% moisture the difference was only 17 units. There were no significant interactions of other variables with protein level.

With regard to the effect of malting conditions, steeping and germination treatments both had a pronounced and approximately equal effect on protease activity. Steeping to the higher level in all cases increased protease activity. However, as is shown in Table X, the response to changes in steeping treatment was conditioned by both protein and germination level. Increasing germination time resulted in higher

protease activity. This effect was consistent except under different steeping conditions as noted above; here the difference was greater when the samples were steeped to 44% moisture. Differences in kilning treatment were consistently without measurable effect.

Relation between Amylase and Protease Activity

The amylase and protease activities of the various malt samples are shown in Table XI. The correlation between these variables for all samples was + .676, a highly significant value since the corresponding 1% point is 0.449. However, the correlation accounted for only 46% of the total variability and inspection of the data in Table XI indicates that the association between amylase and protease activity was markedly affected by the various factors under investigation.

TABLE XI

MEAN VALUES FOR AMYLASE AND PROTEASE ACTIVITIES OF MALTED WHEAT FLOURS

	Hard red spring				Amber durum			
	High protein		Low protein		High protein		Low protein	
Treatment	Amylase	Protease	Amylase	Protease	Amylase	Protease	Amylase	Protease
	Units	*Units*	*Units*	*Units*	*Units*	*Units*	*Units*	*Units*
111	4.53	231	3.43	150	3.40	246	3.76	177
112	4.52	205	3.72	168	3.39	228	3.56	206
211	3.18	113	3.28	66	2.11	130	2.22	138
212	3.35	108	3.48	60	2.16	113	2.48	136
121	1.15	122	0.65	72	1.32	144	1.12	86
122	0.82	115	0.66	69	1.14	151	0.94	84
221	0.74	65	0.74	49	0.77	123	0.66	83
222	0.64	62	0.65	42	0.74	98	0.75	100
Mean all treatments	2.367	127.7	2.074	84.5	1.880	154.2	1.938	126.3

The most practical method of evaluating the relationship between these two types of activity seemed to be to study the variation in protease activity of quantities of malt so selected as to give a constant level of amylase activity. Accordingly, the dosage of each malt required to produce a constant level of gassing power (204.7 ml. in 5 hours at 30° C. per 14 g. flour with 3% yeast) was calculated. This gassing-power level with the base flour employed was found to be equivalent to 280 mg. maltose per 10 g. flour, as determined by the Blish and Sandstedt method. These calculated dosages are given in the second column of Table XII.

In order to test the accuracy of this technique, these amounts of malt flour were added to the same base flour used throughout the investigation and duplicate determinations of gassing power were made. The mean value for all blends was 205.5 ml. and the variation between blends was not significantly greater than the duplicate error.

TABLE XII

MALT DOSAGES TO GIVE CONSTANT GASSING POWER AND PROTEASE ACTIVITY UNITS CONTRIBUTED

Malting treatment	Malt dosage to give 204.7 ml. CO_2, %	Protease activity	
		Units per 100 g. malt	Units contributed by malt at constant gassing power
HARD RED SPRING—HIGH PROTEIN			
111	0.229	231	0.53
112	0.246	205	0.50
211	0.352	113	0.40
212	0.298	109	0.32
121	0.879	122	1.07
122	1.171	115	1.35
221	1.398	65	0.91
222	1.552	62	0.96
HARD RED SPRING—LOW PROTEIN			
111	0.302	150	0.45
112	0.304	168	0.51
211	0.310	66	0.20
212	0.282	60	0.17
121	1.530	72	1.10
122	1.538	69	1.06
221	1.354	49	0.66
222	1.588	42	0.67
AMBER DURUM—HIGH PROTEIN			
111	0.318	246	0.78
112	0.314	228	0.72
211	0.462	130	0.60
212	0.492	113	0.56
121	0.770	144	1.11
122	0.870	151	1.31
221	1.298	123	1.60
222	1.308	98	1.28
AMBER DURUM—LOW PROTEIN			
111	0.302	177	0.53
112	0.290	206	0.60
211	0.450	138	0.62
212	0.398	136	0.54
121	0.932	86	0.80
122	1.037	84	0.87
221	1.392	83	1.16
222	1.382	100	1.38

The third column of Table XII shows the mean protease activities of each malt and the last column shows the calculated protease activity contributed by the quantities of malt required to produce the constant level of gassing power referred to above.

The average effect of malting treatments for the four wheats on protease activity at constant amylase activity is given in Table XIII and the

TABLE XIII

AVERAGE EFFECT OF STEEPING, GERMINATION, AND KILNING TREATMENTS ON PROTEASE ACTIVITY AT CONSTANT AMYLASE ACTIVITY

Treatment	Steeping moisture	Germination	Kilning temp.	Protease activity
	%	*Days*	°F.	*Units*
111	44	5	100	0.572
112	44	5	100–130	0.582
211	40	5	100	0.455
212	40	5	100–130	0.398
121	44	3	100	1.020
122	44	3	100–130	1.148
221	40	3	100	1.082
222	40	3	100–130	1.072

TABLE XIV

AVERAGE EFFECT OF WHEAT TYPE AND PROTEIN CONTENT ON PROTEASE ACTIVITY AT CONSTANT AMYLASE ACTIVITY

Wheat type	Wheat protein	Protease activity
	%	*Units*
Hard red spring	14.3	0.755
Hard red spring	12.1	0.602
Amber durum	14.2	0.995
Amber durum	12.8	0.812

TABLE XV

AVERAGE EFFECT OF WHEAT TYPE, PROTEIN CONTENT, AND MALTING CONDITIONS ON PROTEASE ACTIVITY AT CONSTANT AMYLASE ACTIVITY

Effect of:	Difference in protease activity
	Units
Wheat type (hard red spring minus amber durum)	−0.225
Increasing protein content	0.168
Increasing steeping level	
a. Hard red spring	0.285
b. Amber durum	−0.128
Increasing germination time	−0.579
Increasing kilning temperature	—

corresponding values for the effect of wheat type and protein content in Table XIV. In order to show these effects more clearly the differences between the two levels of each factor are given in Table XV, only statistically significant values being shown. From these comparisons, a lower level of protease activity for a given level of gassing power would result from the use of low-protein hard red spring wheat steeped to 40% moisture and germinated for five days.

Baking Tests

In order to ascertain whether the variations in proteolytic activity discussed above, or other undetermined factors, would affect the baking characteristics of flours diastated with the various malts, blends were prepared using the quantities of the different malt flours shown in Table XII. Thus a series of flour blends of equal gassing power, as proved by actual test, was obtained. These blends were baked by the technique previously outlined, which involved the use of 5% sugar and a four-hour fermentation time. A fermentation time longer than that of the A. A. C. C. basic procedure was used to provide a greater opportunity for detecting the effect of variations in protease activity or other factors. In order to preclude the possibility that a deficiency in gassing power might influence the results under the longer fermentation conditions, 5% sucrose was added. The flours were baked in triplicate in random order.

The mean loaf volume for all flours and replicates was 586.0 cc. The variation within replicates was not large (standard error of single determination = 20.2 cc.) yet the variation between flours was less than 10% of this replicate variability. Obviously, therefore, the variations in proteolytic activity were without influence on loaf volume. No differences in external or internal characteristics of the bread or in the dough handling properties were detectable and it may therefore be concluded that under the conditions of these experiments, factors other than amylase activity were without appreciable influence on the value of the malt for diastating purposes.

Discussion

This project was undertaken primarily to secure an estimate of the relative importance of the various factors investigated and to serve as a guide for possible future intensive studies. In such a preliminary survey it was practical to study each variable at two levels only, and hence the conclusions drawn from this investigation must necessarily be regarded as tentative since the effect of any variable is doubtless de-

pendent upon the range over which it is studied. Despite these limitations, certain aspects of the results obtained seem worthy of comment.

The results of the baking tests clearly indicate that the amylase activity of the malted wheat flours is the only property of any appreciable significance in their utility for diastating purposes. The most important aspect of the study is, therefore, a consideration of the relative significance of the various factors which affect amylase activity. Of the several variables studied, germination time was by far the most important, accounting for 82% of the total variance. In contrast, steeping level accounted for only 5.7%, wheat type 1.4%, and protein content 0.2%; within the limits of the treatments applied, kilning was without any measurable effect. The remaining 11% of the total variance is ascribable to interactions and experimental error. As was previously indicated the relative magnitudes of these effects might be somewhat different had other levels been used in testing the respective factors. The malting conditions which produced the highest amylase activity—germination for five days and steeping to 44% moisture—also gave the lowest yield of malt. This loss, however, is of little consequence when compared with the increase in activity. Thus the mean activity for all wheats malted under the above conditions was five times that of the malts produced by steeping to 40% moisture and germinating for three days. The malting loss under the former conditions was 6.8% as against 2.0% for malts produced at the lower steep and shorter germination.

Since wheat type and protein level were without effect on malting loss, these variables may be considered entirely on the basis of their ability to produce malts of high amylase activity. Our studies show that hard red spring wheats give somewhat higher amylase values than durum; however, in no case was the difference large and the relative behavior of the two wheat types was not consistent at the two protein levels and for different malting treatments. This suggests that at different protein levels and under other malting conditions, the relative amylase activities of hard red spring and durum malt flours might well be different from those encountered in this study. The evaluation of wheat type might profitably be investigated over a range in protein content under fixed germination and optimum steeping conditions.

In regard to protease activity, the baking tests indicate that this property is not of appreciable importance with the samples studied under the specific malting conditions employed. It has previously been reported, for example by Sherwood and Bailey (1926a, 1926b), that increasing amounts of malted wheat had a deleterious effect on the baking quality of flours milled from blends with ungerminated material, particularly in the instance of wheats germinated for five days. These

authors suggest that the undesirable effects may have been caused by excessive proteolytic activity. Our findings are not in accord with this conclusion since our data indicate that when the proteolytic-activity values of the malts are compared on the basis of the quantities of malt required to produce a uniform gassing rate, the proteolytic activity of the five-day malts is actually only about one-half that contributed by the three-day malts. This method of estimating the relative proteolytic activity at constant gassing power conforms most closely to the commercial application of malted wheat flour for diastating purposes and therefore seems the most logical approach. It appears probable that the deleterious effects noted by Sherwood and Bailey were due to factors other than proteolytic activity. In view of the fact that these investigators report results based on the addition of amounts of germinated wheat of from 5% to 40% of the total, the harmful effects noted may have been due to excessive starch liquefaction.

It would indeed be surprising if the variations in proteolytic activity due to the addition of the various malt flours tested in the present study were found to be of appreciable significance. The proteolytic activity of the base flour to which the malts were added was 53 units per 100 g., as determined by the Ayre and Anderson technique (1939), while that contributed by the malt flours at the dosages employed varied from approximately 0.2 to 1.6 units; hence, the differences in the proteolytic activity thus contributed were negligible in relation to the total. Accordingly it may be concluded that, unless conditions widely different from those of this study are employed, and unless under these different conditions the ratio between amylase and protease activity is markedly changed, protease activity cannot be a factor of any material importance in the quality of malted wheat flours.

Summary

Representative composite samples of high-grade hard red spring and amber durum wheats, each at two protein levels differing by approximately 2%, were experimentally malted using eight different treatments representing combinations of steeping to 44% and 40% moisture content, germinating for five and three days, and kilning for 12 hours at 100° F. and for 12 hours at 100° to 130° F.

Growth and yield of malt were not affected by wheat type or protein content. Increasing the steeping level and germination time resulted in greater growth and lower yield; raising the kilning temperature decreased yield slightly.

Protein content of malted wheats and of flours experimentally milled therefrom decreased with conditions which tended to increase growth,

the effect being greater for flours. In general, decrease in ash was not significant.

Amylase activity, expressed as the reciprocal of the amount of malt required to produce constant gassing power and constant autolytic maltose production in blends with a common base flour, was influenced by all factors investigated except kilning. Increase in germination time and steeping level and the use of hard red spring wheat rather than durum raised amylase activity. The over-all mean difference between levels for these factors was 2.44, 0.64 and 0.31 units respectively. However, the difference between levels for any one factor was affected by variation in other factors. The highest activity was obtained with high-protein hard red spring wheat steeped to 44% moisture and germinated five days.

Autolytic protease activity of the malted wheat flour was increased with longer germination, higher steeping level, higher protein, and with durum wheat, the over-all mean difference between levels being 63.1, 60.5, 35.5 and 34.1 units respectively. The effect of steeping was conditioned by protein level and germination time; conversely the last two factors were influenced by steeping level.

Amylase and protease activity were significantly correlated ($r = +.676$). The ratio between these activities was markedly influenced by wheat type, protein content, steeping level, and germination time. At constant amylase activity, the lowest protease activity was obtained with low-protein hard red spring wheat steeped to 40% moisture and germinated five days.

Baking tests on blends of a common base flour with amounts of the various malt flours giving constant and normal gassing power revealed no significant difference in dough handling properties, loaf volume, and external and internal bread characteristics. Unless malting conditions exist which would produce a widely different ratio between amylase and protease activity than those encountered in this study, it would appear that protease activity is of little or no significance in evaluating wheat malts for diastating purposes.

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THE APPLICATION OF THE DROPPING MERCURY ELECTRODE TO THE STUDY OF OXIDATION-REDUCTION SYSTEMS IN FLOUR

H. A. LAITINEN and B. SULLIVAN

Russell-Miller Milling Company, Minneapolis, Minnesota

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It was hoped that the polarograph would offer a valuable tool in the study of any possible oxidation-reduction systems present in flour. The polarographic method involves the determination of current-voltage curves in electrolysis experiments by means of the dropping mercury electrode. The polarograph makes possible a photographic record or polarogram of the current-voltage curve, which is obtained automatically by recording the deflection of a current-reading galvanometer in series with the electrolysis cell, across which a continually increasing e.m.f. is applied. For each reducible or oxidizable sub-

stance present in solution a characteristic "wave" is obtained on the polarogram.

Each wave consists of a region in which the current rises rapidly and levels off to a region of diffusion current, that is, a region of potentials over which the current is practically constant, and is determined by the rate of diffusion of reducible or oxidizable material to the surface of the dropping mercury electrode. In general, the diffusion current is proportional to the concentration of material undergoing electrode reaction. In a given electrolysis medium many oxidation-reduction systems are characterized by constant "half-wave potentials." Thus the potential at which the current has reached one-half of the diffusion current is determined by the nature of the electrode reaction and is independent of the concentration of diffusing material. For the theory of polarography and the interpretation of polarograms in general, reference should be made to the review papers of Kolthoff and Lingane (1939) on inorganic polarography and Müller (1939) on organic polarography.

Experimental

The electrolysis cell and dropping electrode were of the type described by Lingane and Laitinen (1939). A saturated calomel reference electrode (S.C.E.) was used in all experiments. Dissolved oxygen was removed by bubbling tank nitrogen through the electrolysis

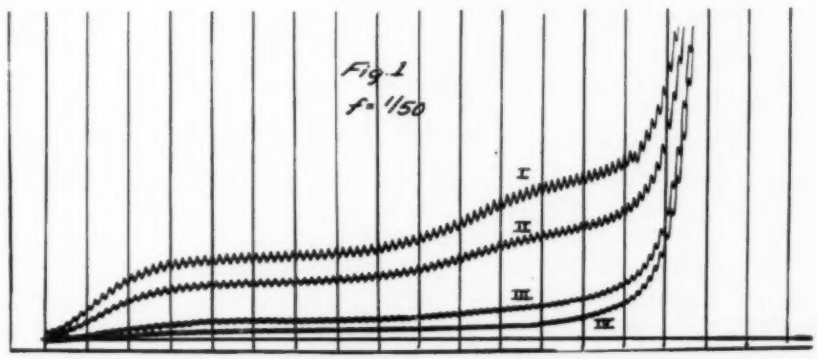

Fig. 1. Oxygen-reduction waves in presence of flour (Curve I) and effect of removal of oxygen by nitrogen (Curves II, III, and IV).

solution for 30 to 45 minutes. Oxygen gives current-voltage curves with two characteristic reduction waves (the first due to reduction to hydrogen peroxide, the second due to the reduction to hydroxyl ions). A series of oxygen-reduction curves is shown in Figure 1. Curve I illustrates a 10% suspension of a straight-grade flour saturated with

air. Curves II, III, and IV illustrate the effect of bubbling nitrogen through the solution for 5, 15, and 35 minutes respectively. The characteristic oxygen-reduction maximum on the curves has been completely eliminated by the presence of the flour.

The galvanometer sensitivity is reported as the fraction of the full sensitivity of 0.0205 microampere per mm. of deflection on the polarogram, and the currents as mm. of deflections at the particular sensitivity used. All curves above the horizontal line represent a reduction (cathodic) process at the dropping mercury electrode while all curves below the horizontal line represent an oxidation (anodic) process at the dropping electrode. In each polarogram, the current-voltage curves begin at an applied e.m.f. of 0.0. Each abscissa division represents an increase of 0.1 volt in the negative potential of the dropping electrode.

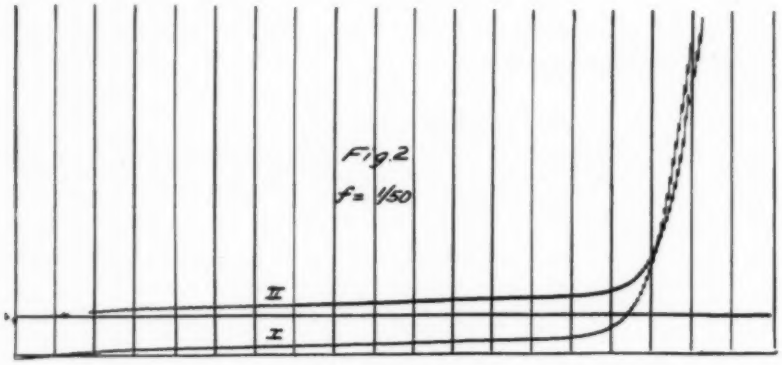

Fig. 2. 0.1*N* KCl extract of a spring-wheat straight.

The simplest extraction possible was first tried on an unbleached spring-wheat straight flour which had shown a strong bromate response. The flour was extracted with 0.1*N* potassium chloride for various lengths of time. Six polarograms were run, of which a typical example is shown in Figure 2. No characteristic waves were found over a range of potentials from + 0.2 to − 1.8 volt (S.C.E.). Since no oxidation-reduction system was detected in the potassium chloride extract, it was thought that an extraction of the flour with an acetate buffer of pH 4.7 would perhaps be more successful. Figure 3, Curve I, shows that no detectable amounts of electro-reducible or oxidizable substances were extracted. A winter-wheat clear was used because it was thought that an unbleached clear would have more reducing substances.

Next the unbleached straight flour was treated with 0.01% papain and then extracted with an acetate buffer. A larger amount of papain

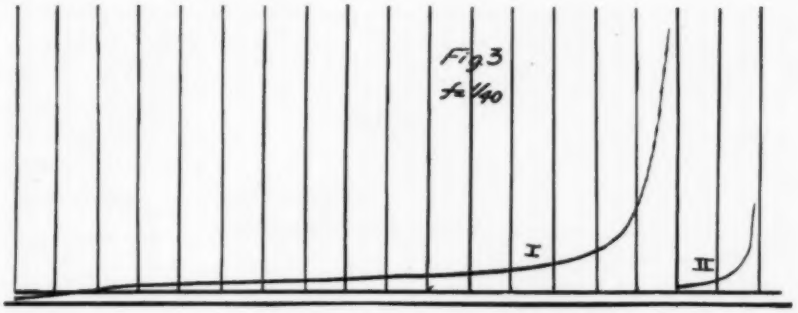

Fig. 3. Acetate buffer extract of winter-wheat clear.

was also tried using for the extraction an acetate buffer of pH 4.7 containing 1% of suspended papain. Both treatments failed to release oxidizable or reducible substances.

Since we knew that glutathione is present in wheat germ, a 0.1*N* potassium chloride extract of the germ was made. A definite anodic wave is seen in Figure 4, Curve I. In such an unbuffered solution, the

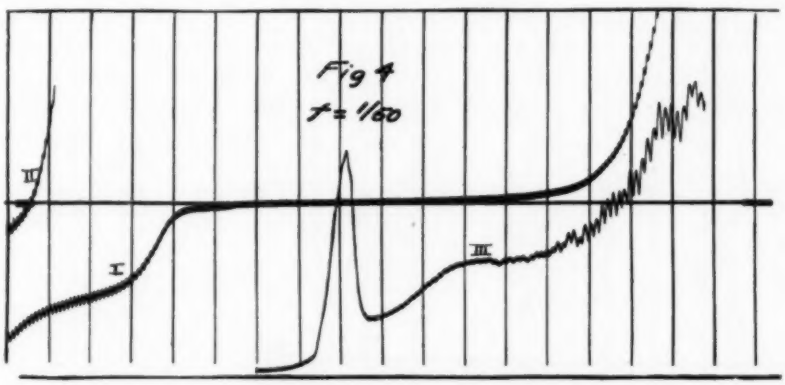

Fig. 4. 0.1*N* KCl extract of wheat germ.

half-wave potential does not have its usual significance. Since hydrogen ions are involved in the electrode reaction the half-wave potential is not constant with changing glutathione concentration, because in unbuffered solution the pH of the solution at the electrode surface is a function of the current. A decreasing pH shifts the anodic wave to more positive potentials. When the germ was extracted in 1*N* hydrochloric acid, or upon acidifying the potassium chloride extract, the polarogram showed no anodic wave, since in a highly acid medium the wave is shifted to such positive potentials that it no longer appears.

Figure 5 shows that the anodic wave is due to glutathione extracted from the germ. Curve I was obtained on an acetate buffer extraction

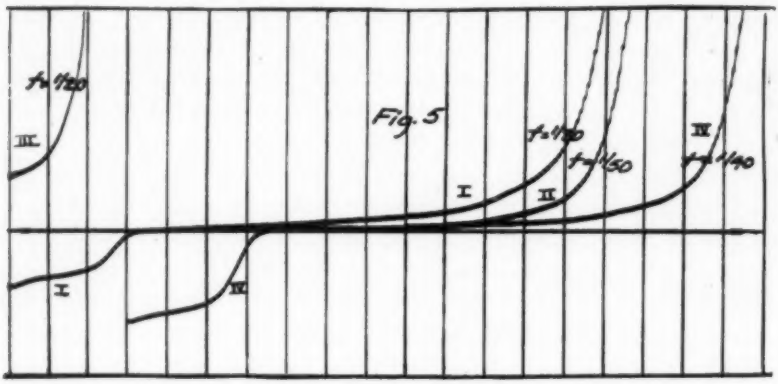

Fig. 5. Acetate buffer extract of wheat germ (Curve I) and after addition of glutathione (Curve II).

of wheat germ and Curve II was obtained after the addition of a small amount of glutathione solution.[1] A smooth curve occurring at the same potential was obtained, indicating that glutathione was responsible for the original wave. The effect of oxidation of the glutathione in wheat germ by treatment with Agene is shown in Figure 6, Curve I.

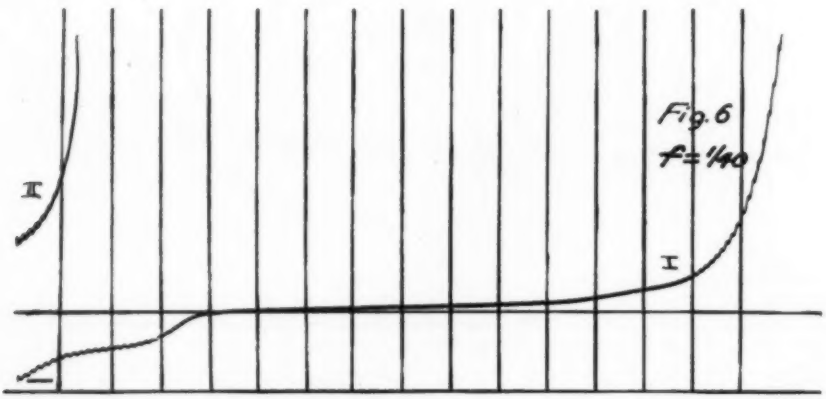

Fig. 6. Effect of oxidation of the glutathione of wheat germ by Agene.

The height of the wave was considerably decreased, but the wave did not entirely disappear. A heat treatment of the germ also decreased the height of the wave considerably.

Since germ but not flour gave an anodic wave using a potassium chloride extract, it was of interest to try the bran. A small amount of material, probably glutathione, was extracted from a winter-wheat bran using 1*N* potassium chloride as is shown in Figure 7, Curve II.

[1] Curves III and IV, Figure 5; Curve II, Figure 6; Curves II–V, Figure 8; and Curves III and IV Figure 11 represent material not reported in this paper.

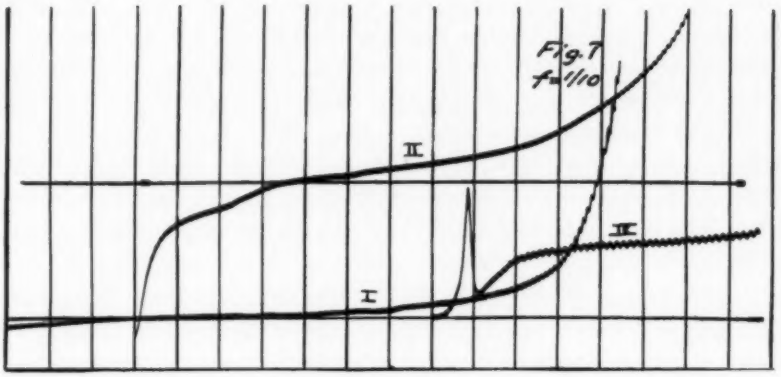

Fig. 7. 1*N* KCl extract of a hard winter wheat bran (Curve II).

The anodic wave disappeared in 1*N* hydrochloric acid medium as in the case of the germ extracts as a result of the high acidity of the medium.

Since we were sure that some reducing substance was present in the flour and since it could not be demonstrated by a simple extraction with an acetate buffer we made an acetate buffer extraction of two doughs (made with yeast, salt, sugar, flour, and water), one with potassium iodate and one without potassium iodate. No distinctive waves were obtained in either case.

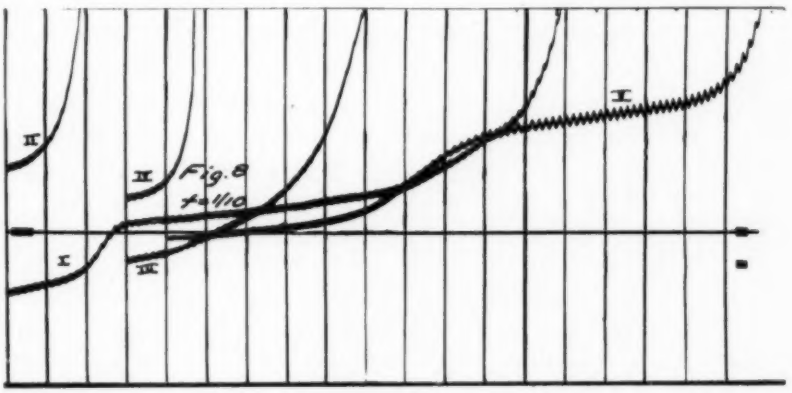

Fig. 8. Acetate buffer extract of yeast (Curve I).

Figure 8, Curve I, however, shows the result of an acetate buffer extraction of yeast. A small anodic wave, probably due to glutathione, was obtained.

Since extractions with potassium chloride as well as with the acetate buffer failed to indicate the presence of a free reducing group in flour,

a more drastic extraction was tried. Various grades and types of flour were extracted for definite times with 1*N* sodium hydroxide. An excess of acetic acid sufficient to make an equimolar acetate buffer was added and the mixture centrifuged. The supernatant liquid was used for the current-voltage curve. An anodic wave with a half-wave potential of − 0.15 volt (S.C.E.) was found. The height of the wave increased with increasing extraction time. After 24 hours of extraction no appreciable further increase in wave height was observed, showing a complete extraction after this period of time. In all experiments reported a 24-hour extraction time was used. Figure 9 shows a comparison of the anodic waves obtained by extracting 20 g.

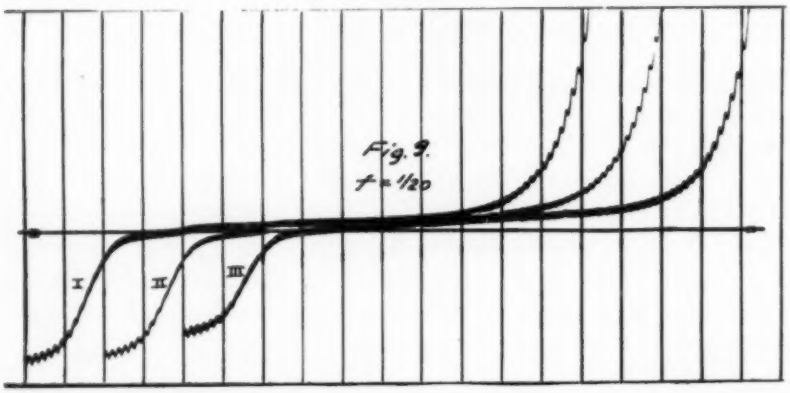

Fig. 9. Comparison of anodic waves of buffered alkaline extracts of patent (Curve III), clear (Curve I), and low-grade (Curve II).

of low-grade (Curve II), clear (Curve I), and patent (Curve III) flours from the same wheat mix, in 200 ml. of 1*N* sodium hydroxide, and buffering 50 ml. of the supernatant liquid with 50 ml. of 2*N* acetic acid. The difference between these particular flours was not very striking, although the patent gave a noticeably smaller anodic wave.

It was shown that the anodic waves were caused by a reducing substance, since the addition of enough potassium iodate to make the solution 2×10^{-4} *M* caused the anodic wave to disappear almost completely. The addition of iodate is discussed more in detail below.

In order to ascertain in what constituent of the flour the reducing substance was present a separation of the starch and gluten was made using a spring-wheat patent and a clear. Two fractions of the starch obtained from both patent and clear by centrifuging were subjected to the alkaline extraction described above. None of the starch fractions showed the presence of a substance giving an anodic wave. On the other hand, alkaline extraction of the wet gluten of flour showed

an anodic wave greatly increased in height over that obtained from the same weight of the whole flour, indicating that all of the material responsible for the wave came from the gluten fraction. An ether-extracted flour gave practically the same anodic wave on treatment with 1*N* sodium hydroxide as the unextracted sample. Likewise the alcohol-ether extract of a dried gluten when treated with sodium hydroxide and buffered showed no reducing substance. Any S-H compound originally present in the fat would probably be oxidized to the S-S form, which is not detectable by a cathodic wave in the presence of substances strongly adsorbed on the mercury surface.

As is the case with the flour itself, the height of the wave was found to depend on the time of alkaline extraction of the gluten. This is evident from Table I, which shows the increasing diffusion current

TABLE I

EFFECT OF EXTRACTION TIME ON HEIGHT OF ANODIC WAVE

Time of extraction of gluten	Diffusion current
Hrs.	*Microamperes*
3.0	3.62
4.5	4.95
24.0	6.23

with increasing extraction time for the wet gluten of a spring-wheat clear.

A comparison of the glutens of patent, clear, and low-grade flours from the same wheat mix is given in Figure 10, Curves I, II, and III.

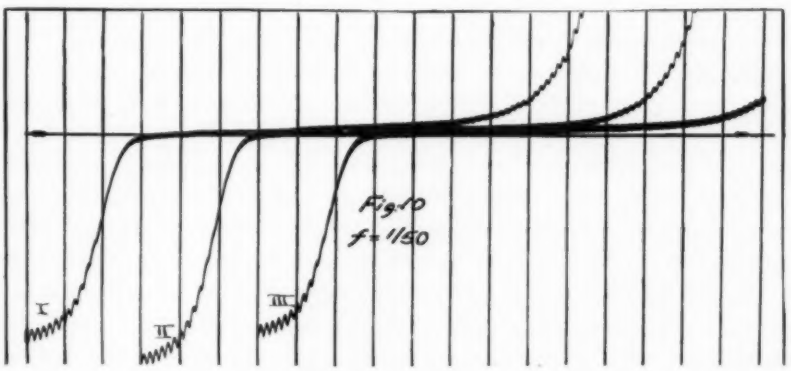

Fig. 10. Comparison of anodic waves of buffered alkaline extracts of gluten from patent (Curve III), clear (Curve II), and low-grade (Curve I).

These curves are based on glutens obtained from equal weights of flour. The results are summarized in Table II. It is to be noted that

TABLE II

COMPARISON OF GLUTENS OF DIFFERENT GRADES OF UNBLEACHED FLOUR

Flour (60 g.)	Wt. of wet gluten extracted	Diffusion current
	g.	*Microamperes*
Low-grade	25.8	5.82
Clear	27.2	6.43
Patent	22.0	5.61

on these particular flours the streams making up the clear and low-grade were such that the clear had a slightly higher protein and wet gluten content than the low-grade flour. The diffusion current is the higher the greater the amount of gluten.

Glutens from a spring-wheat clear flour which had been treated with Agene were compared with those from the unbleached flour. The results are given in Table III. Thus a slight decrease in wave

TABLE III

EFFECT OF AGENE ON HEIGHT OF WAVE IN GLUTEN EXTRACT

Amount of Agene	Diffusion current
g.	*Microamperes*
none	6.23
10	6.02
20	5.71

height, but not nearly as large as one would expect, was obtained with Agene treatment.

Extractions of gluten were attempted using various dispersing agents in order to determine if the reducing substance could be released in any other way than by extraction with 1*N* sodium hydroxide. The solutions used were: 10% sodium salicylate, 10% urea, 30% urea, 2*N* acetic acid, 1*N* sulfuric acid, a borate buffer of pH 10, and a 3% yeast suspension. None of these reagents brought out a reducing substance giving an anodic wave. A complete hydrolysis to the amino acids by boiling for eight hours with 10% sulfuric acid, neutralizing and buffering, likewise failed to give a distinctive wave.

The effect of a treatment of flour with potassium iodate was of interest, since it was thought that the oxidized form of a reducing substance in the flour might give a distinctive cathodic wave on the current-voltage curve. Potassium iodate was chosen rather than potassium bromate since on the dropping mercury electrode, bromate is reduced at much more negative potentials than iodate, and it was desired to determine whether any of the oxidizing agent was reduced

after various times of standing from the height of its polarographic wave.

Figure 11 shows the waves obtained with the equal concentrations of potassium iodate in an equimolar acetate buffer in the absence (Curve I) and the presence (Curve II) of a 10% suspension of a spring-wheat clear flour after centrifuging. In the presence of the flour, the iodate wave was shifted to much more negative potentials, and the diffusion current was considerably decreased. The wave obtained in the presence of flour was shown to be caused by iodate, since the addition of more iodate simply caused the wave height to increase proportionally. The decrease in wave height did not appear to be caused by the reduction of part of the iodate, since no reduction products of iodate appeared. Thus iodine was found to give a cathodic wave beginning at − 0.1 volt (S.C.E.) in the presence of flour, and iodide

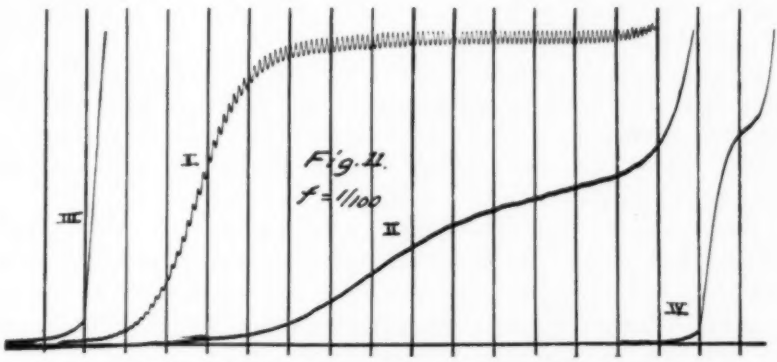

Fig. 11. Potassium iodate curves in acetate buffer in absence (Curve I) and presence (Curve II) of a centrifuged flour suspension.

gives a characteristic anodic wave beginning at about the same potential. Furthermore, the time of standing did not affect the height of the iodate, indicating that no slow reactions occurred.

In order to determine whether the decreased height of the iodate wave in the presence of flour was caused by a simple viscosity effect of the medium, the diffusion current of a metal ion was measured in the presence and absence of a flour suspension. Thallous ions were chosen because of their low tendency to form complexes. Figure 12 shows the polarographic waves of 10^{-3} M thallous chloride in an equimolar acetate buffer in the absence (Curve I) and presence (Curve II) of a 10% centrifuged flour suspension. The diffusion current was changed less than 1% and the half-wave potential was identically the same in the two cases. It may be mentioned that a 2% change in viscosity would be necessary to make a 1% change in the diffusion current, since the diffusion current varies as the square root of the

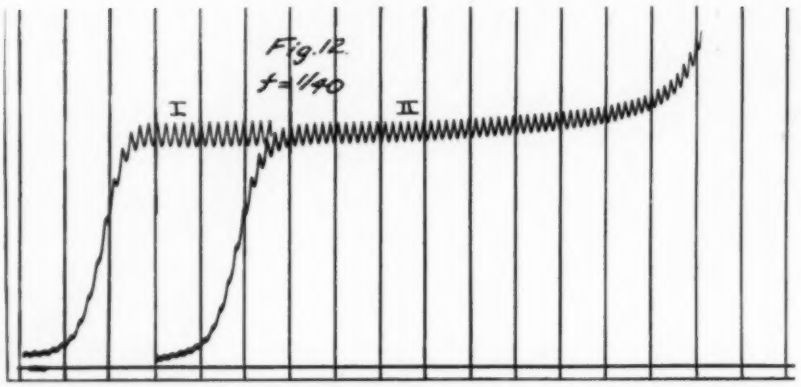

Fig. 12. Thallous chloride (10^{-3} *M*) in acetate buffer in absence (Curve I) and presence (Curve II) of a centrifuged flour suspension.

diffusion coefficient. The viscosity and diffusion coefficient are approximately reciprocally related. The decrease in the height of the iodate wave appears to be due to the formation of complexes between some component of the flour suspension and iodate or some electrode reduction products of iodate. In the former case, the diffusion coefficient of iodate would be affected, while in the latter case a complete reduction to iodide at the electrode could not occur.

To determine whether some of the common amino acids would yield anodic waves by caustic extraction, cystine, methionine, glutamic acid, glycine, tyrosine, and arginine were allowed to stand for several hours in 1*N* sodium hydroxide and the solution buffered as above. In no case was an anodic wave obtained. It is interesting in this connection that cystine gives a cathodic wave in an acetate buffer of pH 4.7 (Figure 14) but in the presence of a flour suspension in the same buffer there is no reduction wave, probably owing to adsorption of the cystine on the flour particles. Pyruvic acid, an intermediate formed during fermentation, gave the reduction wave shown in Figure 13 in an equimolar acetate buffer. Treatment of pyruvic acid with 1*N* sodium hydroxide and subsequent buffering with acetic acid gave identically the same current-voltage curve.

It was of interest to secure some information as to the mechanism of the anodic waves described above in order that we might have some indication of the possible nature of the substance responsible for the wave. In general two different types of mechanisms are possible for anodic waves at the dropping mercury electrode. Anodic waves may be caused by the formation of stable mercurous ion complexes which may be soluble or insoluble. For example, iodide ions give anodic waves due to the formation of mercurous iodide. In such cases no true oxidation of the diffusing substance occurs. On the other hand, the

dropping electrode may act only as an exchanger of electrons as in the oxidation of hydroquinone. In such cases, mercury does not take part in the electrode reaction. If a stable, soluble mercury complex is formed, a detectable amount of mercury is present in solution after prolonged electrolysis with large electrodes.

Tests for complex formation were made by applying a potential corresponding to a point on the diffusion current region of the anodic wave (0.0 volt S.C.E.) to a pool of mercury in one side of a suitable electrolysis cell containing a solution which gave an anodic wave. The

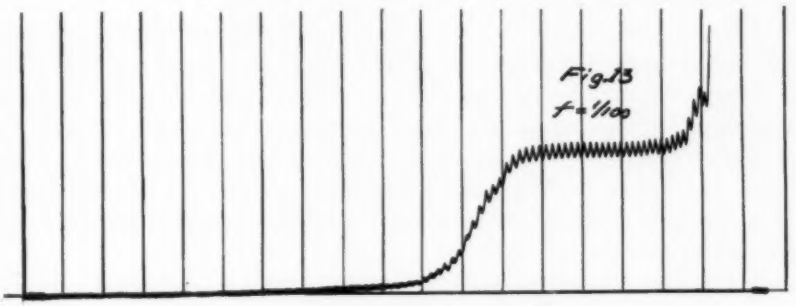

Fig. 13. Pyruvic acid in acetate buffer.

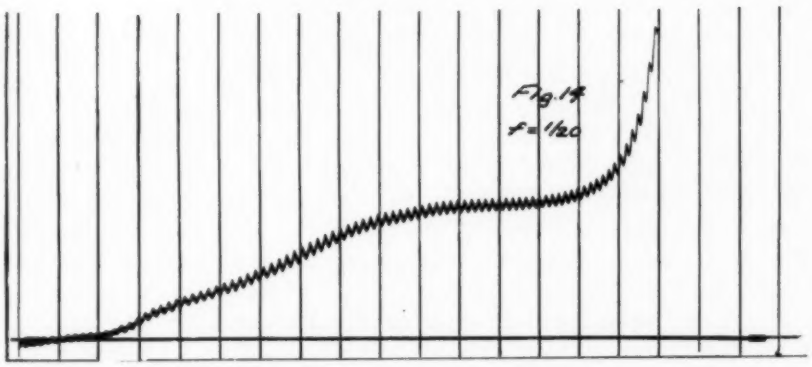

Fig. 14. Cystine in acetate buffer.

other side of the cell, separated by a sintered glass disk, contained 0.1*N* potassium nitrate solution into which was introduced an agar salt bridge from a saturated calomel electrode. The air was removed from the solution to be tested by bubbling nitrogen through it. The saturated calomel electrode and the pool of mercury were short-circuited for several hours with the continual passage of nitrogen for stirring. The solution was tested for the presence of mercury by shaking a 10-ml. portion of it with 5 ml. of 0.001% diphenylthiocarbazone in carbon tetrachloride. A characteristic orange color showed the presence of mercury.

Positive tests for mercury were obtained after electrolyzing the following solution: cysteine in an acetate buffer, extract of wheat germ in an acetate buffer, and the buffered solution containing an alkaline extract of gluten.

In the case of cysteine and glutathione, the positive mercury tests indicate that the anodic waves are caused by the formation of mercury complexes with the -SH groups of the type RSHg. The reducing substance released from gluten by sodium hydroxide also forms a stable mercury complex, an observation which might indicate a sulfur compound was responsible.

Our previous work (Sullivan, Howe, Schmalz, and Astleford, 1940) has indicated quite definitely that some grouping present in the gluten of flour is responsible for the action of certain improvers whether they are oxidizing agents or reducing substances causing an oxidation-reduction system. The theory of Jørgensen and of Balls and Hale that the inhibition of the proteolytic enzymes explains the beneficial effect of substances such as bromate or nitrogen trichloride does not seem tenable to us.

Doubtless the proteases of flour are of the papain type and are activated and inhibited by the same reagents as papain but we feel that the action of the proteolytic enzymes present in sound normal flour is of a great deal less importance than is that of the reducing groups liberated by the gluten itself during fermentation.

Unfortunately, in the present study, we were unable to release these reducing groups by any means other than normal sodium hydroxide. It was difficult also to do much with a fermented dough. The limitations of the mercury electrode are such that it is difficult to prove, in the case of dough, the presence of a reducing substance during fermentation since complex formation of certain substances occurs with the flour or dough.

Recent experiments have shown that treatment of the sodium hydroxide extract of gluten with hydrogen sulfide, buffering and removing the hydrogen sulfide with nitrogen or hydrogen greatly increased the height of the anodic wave, indicating the liberation of a greater amount of reducing substance. With this hydrogen sulfide treatment it was found that approximately one quarter of the reducing substance came from the lipids and about three quarters from the remainder of the gluten.

Conclusion

A substance giving an anodic wave (indicating a reducing substance) was released from the gluten on treatment with sodium hydroxide but not from the starch. A variety of other treatments failed to liberate

the substance. A buffered solution containing the alkaline-treated wet gluten gave a positive test for mercury on electrolysis, indicating that there was formation of a mercury complex with the material in question. Certain RSH compounds give similar mercury complexes. The exact nature of the group giving that anodic wave is not known but indications are that it may be some reduced sulfur linkage.

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SELENIUM IN WHEAT AND WHEAT PRODUCTS

H. W. LAKIN and H. G. BYERS [1]

Division of Soil Chemistry and Physics, U. S. Department of Agriculture, Washington, D. C.

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For many years there has been known a livestock disorder which occurs in parts of the semiarid Great Plains of the United States. This disorder is known in the plains as "alkali disease," a misnomer applied by early settlers who attributed the trouble to alkali (high salt) water. The disorder is now known to be selenium poisoning and is caused by the ingestion of vegetation that has absorbed this toxic element from the soil (Beath, Draize, and Gilbert, 1934; Byers, 1936; Franke, Rice, Johnson, and Schoening, 1934). It has been found that relatively small amounts of selenium taken daily disturb the physiological processes. Rats on an adequate diet prepared to contain 6 ppm. (parts per million) of selenium, by the incorporation of seleniferous cereals, were found to be considerably below normal in weight. Furthermore the number of young born and the percentage reared were less than normal. A similar diet containing 3 ppm. of selenium had a slight effect on reproduction, although growth was apparently normal (Munsell, De Vaney, and Kennedy, 1936). During field studies in affected areas we have observed selenized animals suffering

[1] Associate Chemist and Principal Chemist.

various gradations of affliction. Pigs have been encountered that were nearly hairless from ingestion of toxic grain, horses without tail or mane, and with deformed hoofs, cows with poor coats and loose hoofs, and bulls impotent from seleniferous forage. These symptoms are accompanied by poor general condition of the affected animals. Farms were encountered where it was necessary to purchase eggs for setting, since the eggs from the flock would not hatch.

Many farmers are familiar with seleniferous "alkalied" grain, and use various methods to circumvent its toxicity. Some farmers sell their grain and buy good grain for feeding. Others feed a mixture of seleniferous grain with good grain from other fields on their own farm or obtained elsewhere.

Because of numerous inquiries concerning the health hazards incurred by people outside of seleniferous areas, it seemed of importance to determine to what extent foodstuffs which may come into the markets are likely to contain significant amounts of selenium. Therefore a comprehensive survey of the grain crops of areas known to be seleniferous was made by one of the authors during the harvest season of 1937. This survey was supplemented by samples furnished by the U. S. Food and Drug Administration and the General Mills Company, Inc. In this manner, 950 samples of wheat and wheat products were obtained, together with about 300 samples of barley, corn, oats, and rye. These samples were analyzed and the results will be given in detail in a technical bulletin of the U. S. Department of Agriculture (Williams, Lakin, and Byers, 1940). It is considered advisable by the authors to make immediately available a résumé of the results obtained from the determination of selenium in the samples of wheat and wheat products.

Wheat and wheat products were collected in portions of Colorado, Kansas, Nebraska, South Dakota, Wyoming, Montana, North Dakota, and Minnesota. The places of collection were in the seleniferous areas or, if outside the areas, in those towns that might handle some grain from such areas. In Table I the counties in which samples were collected are listed for each state involved in the survey except Minnesota. The six samples obtained from this state were obtained at grain centers believed to handle grain from seleniferous areas farther west. Areas of soils capable of producing seleniferous grain are small and are interspersed with soils of much lower selenium content. The percentage of the land in the counties listed in Table I, known or thought to be seleniferous, varies over a wide range, but in no case could it be said that all of the soils in any of these counties are seleniferous.

TABLE I

DISTRIBUTION OF POINTS OF COLLECTION BY COUNTIES WITHIN THE STATES

State	Counties		
Colorado	Bent	Otero	Rapahoe
	Crowley	Prowers	
Kansas	Gove	Logan	Rooks
	Graham	Ness	Trego
	Lane	Phillips	Wallace
Montana	Big Horn	Glacier	Roosevelt
	Blaine	Hill	Sheridan
	Carter	Judith Basin	Teton
	Cascade	Liberty	Toole
	Choteau	Petroleum	Valley
	Daniels	Phillips	Yellowstone
	Fergus	Pondera	
Nebraska	Boyd	Harlan	Red Willow
	Dawes	Hitchcock	Sheridan
	Furnas	Knox	Sioux
North Dakota	Burleigh	Ward	Williams
	Mountrail		
South Dakota	Brule	Haakon	Meade
	Butte	Hughes	Pennington
	Custer	Jackson	Stanley
	Fall River	Jones	Tripp
	Gregory	Lyman	
Wyoming	Albany	Crook	Niobrara
	Big Horn	Goshen	Platte
	Campell	Johnson	Sheridan
	Converse	Natrona	Washakie
			Weston

Samples of wheat were collected primarily at elevators in shipping centers. Wheat and wheat products were obtained from flour mills. Also, samples were collected from fields, storage bins on farms, and from trucks hauling from farms. It naturally follows that the individual samples are representative of various sources of wheat ranging in quantity from a few bushels to many thousand.

Discussion of Data

Of the 951 samples examined, 82.5% contained 1 ppm. or less, 10% contained 2 to 3 ppm., and 7.5% contained 4 ppm. or more of selenium. Only 8 samples contained as much as 10 ppm. or more of selenium. A more detailed analysis of the data by states is summarized in Table II.

Since there is little in the literature which permits an appraisal of the selenium intake tolerated by human beings, without injury, all references to toxicity in this discussion relate to experimental or domestic animals. Certainly those samples that contain 4 ppm. or

TABLE II

DISTRIBUTION OF 951 WHEAT AND WHEAT PRODUCT SAMPLES FROM SELENIFEROUS AREAS IN EIGHT STATES, ARRANGED ON THE BASIS OF SELENIUM CONTENT

Location	Total number of samples	Percentage distribution on basis of selenium content						
		0.1 ppm. or less	0.2 ppm.	0.5 ppm.	1 ppm.	2 ppm.	3 ppm.	4 ppm. or more
		%	%	%	%	%	%	%
Colorado	16	6	6	25	44	6	13	0
Kansas	61	1.5	6.5	36	26	20	5	5
Montana	410	15.9	25.6	35.4	19.5	3.2	0.2	0.2
Nebraska	106	13.2	16.0	21.7	19.8	8.5	8.5	12.3
North Dakota	84	3.6	29.8	39.3	22.6	3.6	1.1	0.0
South Dakota	188	2.1	11.2	14.5	15.4	15.9	10.6	30.3
Wyoming	80	18.7	31.2	40.0	8.8	0.0	1.2	0.0
Minnesota	6	0.0	67	33	0.0	0.0	0.0	0.0

more, if they constitute the whole diet, would be toxic to white rats. From this viewpoint the vast majority of the samples examined cannot be considered toxic. Only those samples collected in seleniferous areas in Nebraska and South Dakota show an appreciable percentage containing 4 ppm. or more. Many of these samples are from farms and represent relatively small quantities of wheat.

In two adjacent towns in a particularly toxic area in South Dakota, 25 samples of wheat were collected from elevator bins and freight cars. Twenty of these samples contained 4 to 8 ppm. of selenium. These samples would be toxic to white rats, and the 14 samples containing in excess of 5 ppm. would adversely affect the hatchability of chicken eggs if used as the total laying ration (Poley and Moxon, 1938).

Flour was collected at mills in South Dakota, Nebraska, Wyoming, Montana, and North Dakota. Of the 66 samples of flour examined, only 5 contained in excess of 1 ppm. of selenium. Two samples of flour from a small roller mill in South Dakota contained 4 and 5 ppm. of selenium. The wheat, bran, middlings, and wheat cleanings collected at this mill also had a selenium content of 4 to 5 ppm. A similar situation was observed in northwestern Nebraska. From a small mill in this area a sample of flour was found to contain 4 ppm., bran 4 ppm., shorts 6 ppm., and wheat 4, 10, and 0.5 ppm. of selenium. One should not infer from these data that these two mills always produce flour containing 4 to 5 ppm. of selenium. These samples merely represent the flour sampled at the time the mills were visited. Other mills in seleniferous areas may occasionally produce flour of higher selenium content than was found in their flour at the time they were visited. The data show that a very small percentage of the flour produced in seleniferous areas contains selenium in excess of the minimum requirement for injury to white rats.

The data used in Table II and discussed above represent wheat and wheat products in, or adjacent to, seleniferous areas. The wheat from these areas constitutes, in all cases with the possible exception of Montana, a minor portion of the wheat production of the states involved. Therefore it is to be emphasized that the percentage distribution shown in the table represents only the wheat produced from portions of the states in which selenium is known to be a possible component in significant quantities. Furthermore, these samples are representative of the grain produced in the United States most likely to be toxic due to selenium, and only 7.5% of the samples contain sufficient selenium to be toxic to white rats when used as the *whole* diet. Since the wheat production of seleniferous areas in the United States is a minor portion of the total crop, one may reasonably presume that the seleniferous wheat is greatly diluted when shipped to large milling centers and that only in mills located in seleniferous areas could one expect to find flour containing significant amounts of selenium.

Selenium has been found in all samples of wheat examined in this laboratory, when sufficient material has been used in the analysis. Robinson (1936) has examined wheat from Argentina, Australia, Canada, Hungary, Mexico, South Africa, and Spain, in addition to domestic samples. He has found selenium in all of the samples examined. The authors agree with Robinson that it is improbable that any field-grown wheat is entirely free from selenium, since, when carefully examined, wheat has always been found to contain selenium. The mere presence of selenium in wheat must not be viewed with alarm since it is probably a normal constituent and becomes a problem only when it occurs in sufficient quantities to be injurious.

Summary

The selenium content of 951 samples of wheat and wheat products from seleniferous areas in the United States was determined and a résumé of the results given. The vast majority of these samples were low in selenium content. Samples of wheat and wheat products containing sufficient selenium to be toxic to domestic animals were found. From the known facts the authors believe selenium to be present in all wheat in detectable amounts.

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DOUGH OXIDATION AND MIXING STUDIES. IV. EFFECTS OF OXYGEN AND POTASSIUM BROMATE IN SPONGE DOUGHS

JACOB FREILICH and CHARLES N. FREY

The Fleischmann Laboratories, Standard Brands Incorporated, New York, N. Y.

(Read at the Annual Meeting, May 1939)

It has previously been reported (Freilich and Frey, 1939) that bromate in straight doughs was found to produce effects, in addition to the inhibition of proteases, which were partly dependent on dough fermentation products. Remixing straight doughs after fermentation overcame certain "excess bromate" effects. Oxygen was found to inhibit protease activity and diminish the harmful effects of wheat germ.

Experimental

In the present study 60%–40% sponges were used with 1.5% yeast in the sponge and 1.67% salt, 5% sugar, and 3% shortening in the dough, with variable absorption. Sponge time was generally 3½ hours at 86° F., dough time 20 minutes, bench proof 15 minutes, pan proof to constant height at 100° F., and oven temperature 410° F. All doughs were scaled to 480 g. and molded by machine. Mixing was done in a specially adapted Hobart-Swanson mixer.

Tests were made to determine the effects of bromate and oxygen on doughs fortified with protease (papain) and wheat germ. Unless otherwise stated these latter ingredients were used at levels of 0.014% and 3% in the sponge and 0.0093% and 2% in the dough, respectively.

Figure 1 shows the action of bromate in counteracting the effects of the added protease. Normal loaves were obtained with bromate dosages of 25–50 mg. per loaf, but a slight excess bromate effect appears at 100 mg. Dough time is an important factor in the appearance of

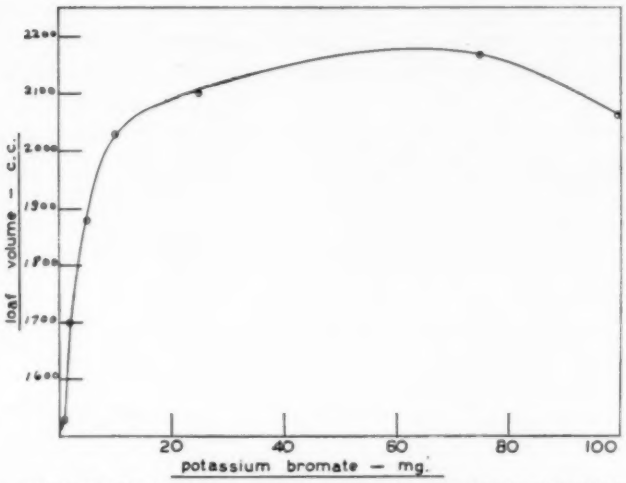

Fig. 1. Effects of potassium bromate on loaf volume of sponge doughs with added papain.

this phenomenon; using 20, 90, and 105 minutes of dough time, loaf volumes of 2,220, 2,060, and 2,020 cc. were obtained with increasing excess-bromate effects in the last two (see Fig. 2). It is to be noted that

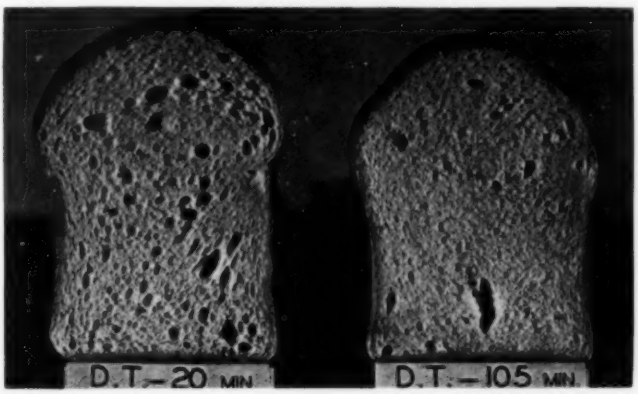

Fig. 2. The " excess bromate " effect in sponge doughs, due to longer dough time (D.T.) after remixing.

the excess-bromate effects here appeared after remixing of the sponges. Since this effect was eliminated in straight doughs by remixing, it is evident that we are dealing with a reversible type of reaction:

$$\text{normal properties} \xrightarrow[\text{remixing}]{\text{bromate + fermentation}} \text{excess bromate effects}$$

Table I and Figures 3 and 4 indicate the similar effect of oxygen, except that an " excess " effect is not obtained. We have not yet ob-

TABLE I

EFFECT OF OXYGEN IN SPONGE DOUGHS

Sponge mixing time	Loaf volume		
	Sponge, N_2 Dough, air	Sponge, O_2 Dough, air	Sponge, O_2 Dough, O_2
min.	*cc.*	*cc.*	*cc.*
1	1610	1660	1650
2	1550	1690	1720
3	1580	1770	1870
4	1560	1900	1960
5	1560	1940	1960
10	—	1970	1990

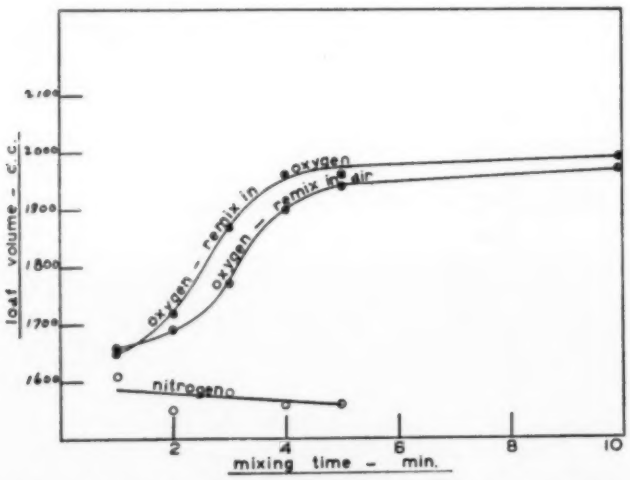

Fig. 3. Effects of mixing in oxygen and nitrogen on loaf volume of sponge doughs with added papain.

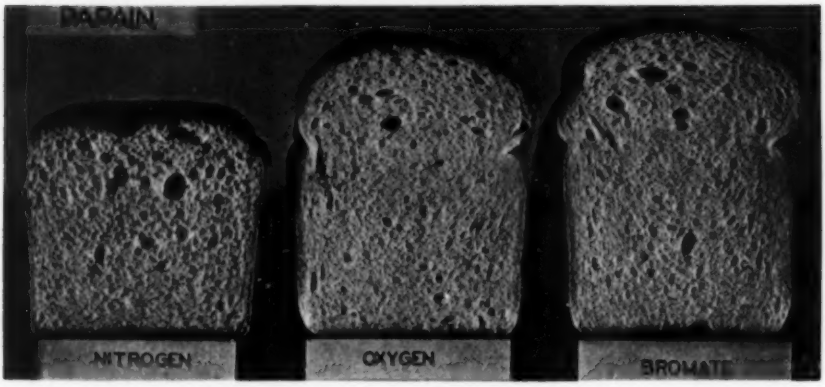

Fig. 4. Sponge doughs with added papain mixed in nitrogen, oxygen, and with added bromate.

tained excess-bromate effects with oxygen even with excessive amounts. Indeed, remixing in oxygen still produces improvement in these cases, indicating that the use of oxygen in the sponge only may not be sufficient for optimum results.

Three samples of wheat germ were tested with the results shown in Table II and Figure 5. Grinding sample C increased its deleterious effect, but oxidation successfully overcame it.

TABLE II

WHEAT GERM

Mixing times: sponge 4 minutes, dough 3 minutes.

Wheat germ	Loaf volume	
	Nitrogen	Oxygen
	cc.	*cc.*
A	2000	2140
B	2050	2150
C	1980	2140
C (ground)	1860	2210
C (ground)	2180[1]	—

[1] 10 mg. $KBrO_3$ in the sponge.

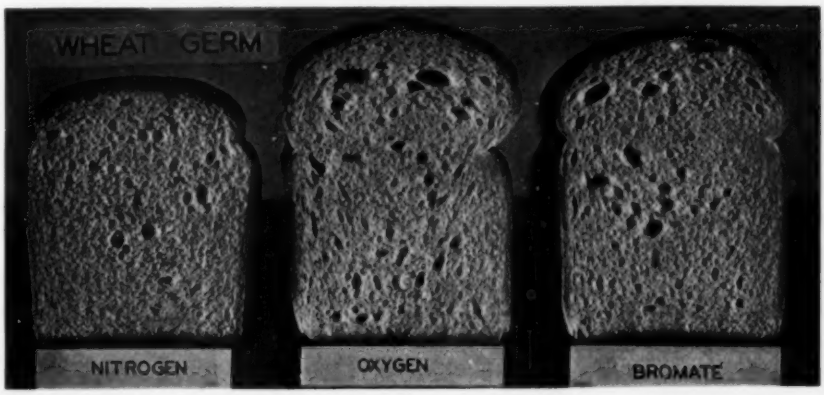

Fig. 5. Sponge doughs with added wheat germ mixed in nitrogen, oxygen, and with added bromate.

That flours differ in their response to oxygen is indicated by the results given in Table III and Figures 6 to 10. In these experiments no added protease or wheat germ was used. Flours No. 7 and No. 8 show slight deleterious effects with oxygen, while this negative response is still more pronounced with flour No. 4. Thus the oxygen requirements of flours may vary over a considerable range. Pan proof times with flours No. 7 to No. 11 and 12 to 14 decreased with a lowering of

TABLE III

EFFECT OF OXYGEN ON VARIOUS FLOURS

No.	Flour	Sponge mixing time	Loaf volume	
			Nitrogen	Oxygen
		min.	*cc.*	*cc.*
1	Southwestern straight	1	1950	1960
	Southwestern straight	5	2000	2160
2	Kansas Standard Patent	1	1910	2020
	Kansas Standard Patent	2	1960	2070
3	Kansas, No. 1	–	1940	2080
4	Kansas, No. 2	–	2070	1890
5	Straight grade, unbleached	–	2000	2160
6	Straight grade, bleached	–	2080	2160
	Northwestern Wheat:			
7	Short Patent	3	2130	2120
8	Long Patent (bleached)	3	2200	2140
9	Straight (bleached)	3	2150	2230
10	Clear	3	2140	2210
11	Low grade	3	1690	2010
	Texas Wheat:			
12	Patent	3	1960	1980
13	Clear	3	1920	2050
14	Low grade	3	1760	2070

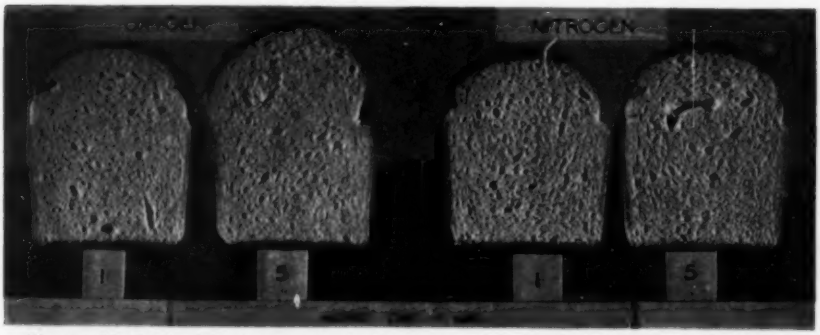

Fig. 6. Effects of mixing in oxygen and nitrogen on sponge doughs made from a Southwestern straight flour.

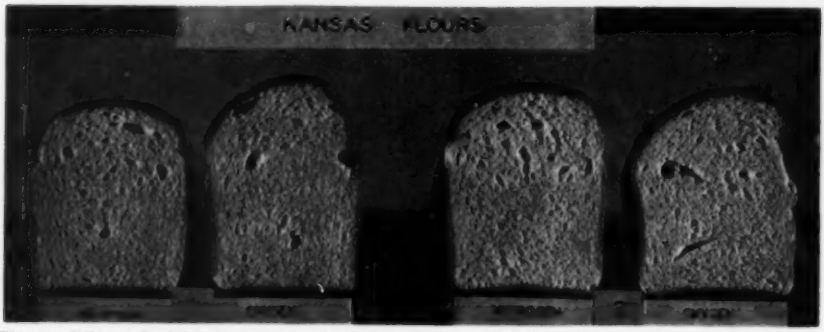

Fig. 7. Effects of mixing in oxygen and nitrogen on sponge doughs with two different Kansas flours.

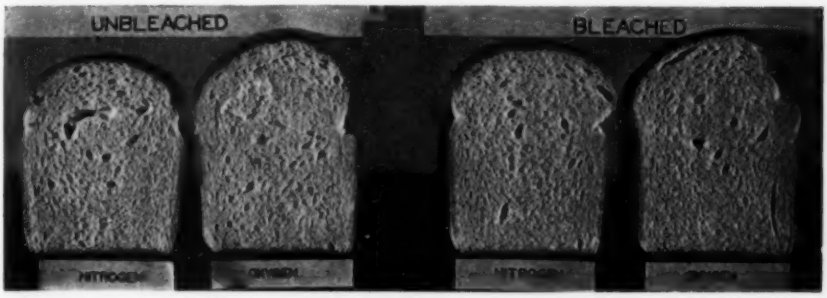

Fig. 8. Effects of mixing in nitrogen and oxygen on sponge doughs made from the unbleached and bleached lots of the same flour.

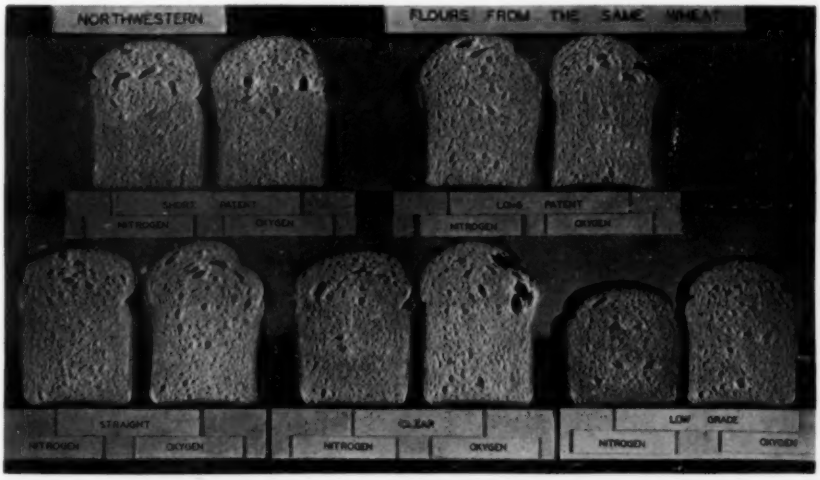

Fig. 9. Sponge doughs made from the different grades of flour that were milled from the same Northwestern wheat, mixed in nitrogen and oxygen.

Fig. 10. Sponge doughs made from the patent, clear, and low grade flours that were milled from the same Texas wheat, mixed in nitrogen and oxygen.

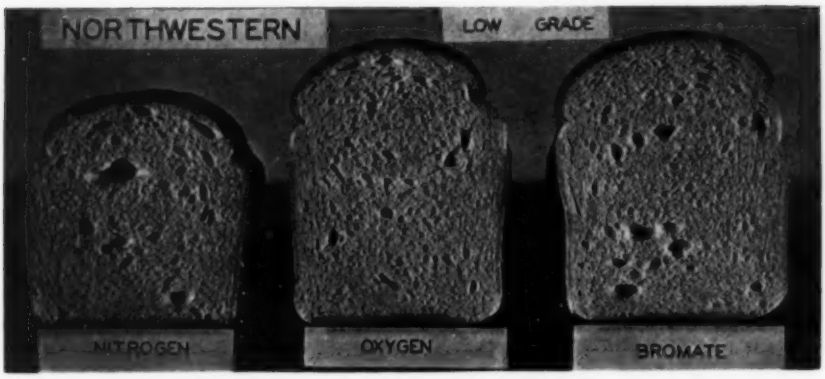

Fig. 11. Sponge doughs with bromate compared to mixing in oxygen and nitrogen, made from Northwestern low grade flour.

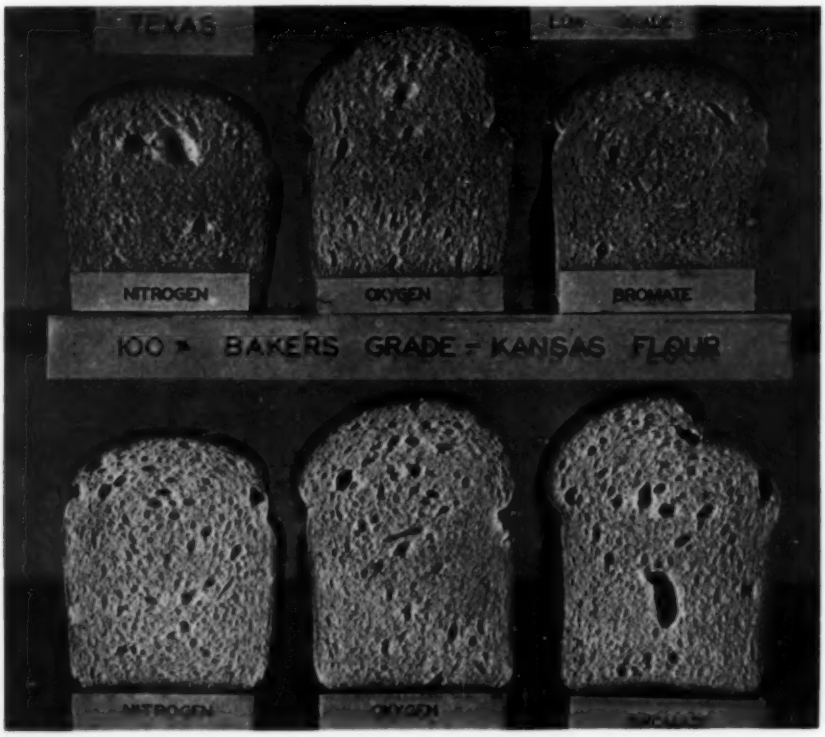

Fig. 12. Sponge doughs with bromate compared to mixing in oxygen and nitrogen, made from Texas low grade and 100% Kansas flours.

the flour grade, an effect particularly pronounced in the case of nitrogen, indicating an increase in soluble nitrogen constituents. These were probably produced partly as a result of increased proteolytic degradation.

Flours also differ in their relative responses to oxygen and bromate, as indicated in Table IV and Figures 11 and 12.

TABLE IV

EFFECTS OF OXYGEN AND BROMATE (10 MG./LOAF) WITH CERTAIN FLOUR TYPES

Flour	Loaf volume		
	Nitrogen	Oxygen	Bromate
	cc.	*cc.*	*cc.*
Northwestern, low grade	1690	2010	2010
Texas, low grade	1760	2070	1800
100% bakers' grade, Kansas	2010	2130	2220

The Texas low grade responds much better to oxygen than to bromate, while the reverse is true with the 100% bakers' grade Kansas flour. These varying results with oxygen and bromate suggest the possibility that their effects in flour may be different, though the final results of their action are similar in many respects.

Discussion

The effects of bromate and of oxygen in sponge doughs with added papain, wheat germ, and with different flours, are found to be generally similar to their effects in straight doughs. There is a difference, however, with respect to the excess-bromate effect. In conventional straight doughs, large amounts of bromate will always produce this effect, but in conventional sponges it will ordinarily not occur, until relatively enormous amounts of bromate are used, because of the remixing after fermentation and the short fermentation time used following the remixing.

There is a difference between ordinary sponges and those mixed in oxygen with respect to mixing time. Ordinarily, the sponge-mixing time may be varied over a wide range without affecting the quality of the resulting bread (Frey, Freilich, and Ekstedt, 1937); for this reason bakers use a very short sponge-mixing period, in comparison to the time of remixing. But when using oxygen alone, a longer mixing time is advantageous in order to incorporate sufficient oxygen for best results. With bromate, a normal sponge-mixing time may be used because it is usually effective even in the absence of oxygen.

The fact that flours differ in their oxygen requirements emphasizes the importance of proper control of the oxidation treatment which they receive.

The difference between the effects of bromate and oxygen with certain flours furnishes some evidence in support of the idea that there may be some fundamental differences between their actions in dough.

Summary

Bromate and O_2 incorporated by mixing counteracted the effects of added protease and wheat germ and improved the bread made from lower-grade flours by the sponge process. "Excess bromate" effects appear only if dough time is greatly prolonged after remixing. Flours vary considerably in their oxidation requirements. Some flours respond better to O_2 than to bromate, while with others the situation is reversed.

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A SIMPLE PHOTOMETRIC METHOD FOR DETERMINING THE PROTEIN CONTENT OF WHEAT FLOUR

LAWRENCE ZELENY [1]

Agricultural Marketing Service, U. S. Department of Agriculture, Washington, D. C.

(Read at the Annual Meeting, May 1940)

The Kjeldahl method, or one of its various modifications, is used extensively for the routine determination of protein in wheat and wheat flour. The method is reliable, fairly rapid, and is well adapted to routine work. Certain shortcomings of the Kjeldahl method, however, definitely limit its usefulness. In order to handle a large volume of work, a protein laboratory must be equipped with elaborate and very expensive equipment which must be permanently installed and is, therefore, nonportable. Large quantities of strong acid and alkali must be used, and noxious fumes and excessive heat are characteristic of many protein laboratories.

The Agricultural Marketing Service is interested in the development of a simplified procedure by which protein determinations may

[1] Associate Grain Technologist.

be made conveniently in small laboratories without the installation of cumbersome equipment. Such a method may be of considerable value in certain types of grain-inspection work.

The method herein described has been developed by utilizing certain physico-chemical properties of the gluten proteins, and is not based on any previously established procedure. It should be mentioned, however, that several colorimetric and turbidimetric methods for determining the protein content of various body fluids have been developed in recent years and are being used to advantage in clinical laboratories for the routine analysis of blood serum, urine, and cerebrospinal fluid. Since the photometer is used with success in some of these clinical methods, it was believed that a photometric method might be developed for the determination of protein in cereal products.

The proteins of wheat flour are readily peptized by very dilute alkali. When an alkaline solution containing the peptized flour proteins is neutralized, the gluten proteins are precipitated. If, however, the protein sol is sufficiently dilute, and its hydrogen-ion concentration after neutralization is controlled by a suitable buffer, the gluten proteins will not separate from the solution but will nevertheless appear as a turbid and stable colloidal suspension. The degree of turbidity of such a suspension is a measure of the gluten protein content of the flour.

Experimental

To determine the optimum hydrogen-ion concentration and the minimum time required for the production of the greatest turbidity in a neutralized alkaline extract of wheat flour, 5-ml. portions of a filtered 0.05*N* potassium hydroxide extract of a flour were added to 25-ml. portions of a series of buffer solutions. The buffers covered a range in pH values from 6.0 to 8.7, and were prepared by mixing M/5 solutions of Na_2HPO_4 and KH_2PO_4 in various proportions. The relative degrees of turbidity of the suspensions produced were determined by measuring the light transmission through a definite depth of suspension by means of a photoelectric photometer, using a light filter with a maximum light transmission at a wave length of 530 millimicrons. The values obtained are given in Table I.

It is evident that under the conditions used the maximum turbidity (minimum light transmission) is attained by using a buffer of pH 7.8, which results in a suspension having a pH of approximately 8.2. It is also evident that the maximum turbidity occurs in about 60 minutes after the addition of the buffer to the extract. To obtain maximum turbidity, however, this length of time does not need to be exact, since the change in turbidity between 45 and 90 minutes is very small. It

TABLE I

TRANSMISSION OF LIGHT AT ±530 mμ. THROUGH SUSPENSIONS PREPARED BY ADDING 5-ML. PORTIONS OF A FILTERED 0.05*N* KOH EXTRACT OF A WHEAT FLOUR TO 25-ML. PORTIONS OF M/5 PHOSPHATE BUFFERS

Suspensions contained in matched 7 × 7/8″ test tubes.

Suspension No.	Buffer formula		H-ion concentration		Light transmission after				
	M/5 Na_2HPO_4	M/5 KH_2PO_4	Buffer	Suspension	15 min.	45 min.	60 min.	90 min.	24 hrs.
	Volumes	*Volumes*	*pH*	*pH*	%	%	%	%	%
1	100	0	8.72	9.63	62.3	60.4	59.9	59.6	63.4
2	99	1	8.39	9.47	57.7	55.6	55.3	55.2	56.7
3	97.5	2.5	8.10	9.10	55.1	53.5	53.2	53.2	53.7
4	95	5	7.86	8.30	52.9	52.0	51.7	51.7	52.8
5	94	6	7.80	8.16	52.3	51.5	51.2	51.3	51.9
6	93	7	7.71	8.02	52.8	51.9	51.6	51.8	52.5
7	92	8	7.63	7.83	53.2	52.0	52.0	52.2	52.8
8	91	9	7.60	7.76	53.5	52.5	52.2	52.3	53.2
9	90	10	7.51	7.62	54.5	53.1	52.7	52.7	53.2
10	80	20	7.23	7.31	55.5	54.3	53.9	53.9	55.3
11	70	30	6.98	7.08	56.7	55.1	55.1	55.1	56.1
12	60	40	6.79	6.88	57.5	56.6	56.5	56.3	57.7
13	50	50	6.61	6.72	60.1	59.4	59.0	58.9	60.6
14	40	60	6.43	6.50	62.7	62.2	61.9	61.8	65.6
15	30	70	6.26	6.36	66.9	66.6	66.4	66.3	86.4 [1]
16	20	80	6.03	6.18	68.9	68.5	68.2	68.2	92.3 [1]

[1] Precipitate settled out.

was further shown that the temperature of the suspensions had very little effect on the final degree of turbidity, at least within the ordinary range of laboratory temperature. For very exact work, however, it is possible that the temperature should be controlled or that a temperature correction factor should be used.

The following method, based on the foregoing experiments, was used to determine the relationship between the turbidity produced and the protein content of the flour:

(1) To exactly 0.5 g. of flour in a 200-ml. Erlenmeyer flask, add exactly 5 ml. of 95% ethyl alcohol (to prevent the flour from coalescing) and 100 ml. of 0.05*N* KOH.

(2) Shake mixture intermittently for 15 minutes and then centrifuge for 10 minutes at approximately 1600 rpm.

(3) To exactly 5 ml. of the centrifugate in a photometer test tube (one of the selected tubes for use in lieu of an absorption cell), add exactly 25 ml. of a buffer solution made by mixing 6 parts by volume of M/5 KH_2PO_4 with 94 parts by volume of M/5 Na_2HPO_4. This buffer solution should have a pH of 7.8. Mix the contents of the test tube by inversion and allow to stand for 1 hour.

(4) Determine the transmission of light through the solution in the test tube with a photoelectric photometer, using a light filter having a maximum transmission at a wave length of 530 millimicrons. (Other wave lengths will give different but equally satisfactory results.)

Thirty-four samples of flour, representing all classes of domestic wheat except durum, were analyzed by this method. These flours ranged in protein content from 7.30% to 16.26%, and in ash content from 0.38% to 0.61%. The results are given in Table II and are shown graphically in comparison with the protein content values as determined by the conventional Kjeldahl method in Figure 1.

TABLE II

SHOWING THE RELATIONSHIP BETWEEN PROTEIN CONTENT AND LIGHT TRANSMISSION VALUES ON A SERIES OF 34 FLOUR SAMPLES

Sample No.	Class and variety of wheat from which flour was milled	Protein content ("as is" basis)		Light transmission at ±530 mμ (in 7 × 7/8" test tubes)
		Total	Not peptized by 5% K_2SO_4	
		%	%	%
1	White (Other than Federation or Baart)	7.30	5.85	70.4
2	White Club	7.66	6.18	69.0
28	White	8.61	6.89	62.8
29	White	8.77	6.98	62.1
3	White Federation	8.80	6.86	65.4
32	Soft Red Winter Purkoff	9.13	7.39	58.7
34	Soft Red Winter Nittany	9.31	7.63	57.6
33	Soft Red Winter Red Rock	9.44	7.59	58.3
4	Hard Red Winter	9.44	7.91	55.9
5	White (Other than Baart)	9.70	8.16	56.7
26	White	9.79	7.96	56.4
6	White Baart	10.09	8.46	55.0
19	Hard Red Spring	10.13	8.46	53.7
10	(Unknown)	10.47	8.57	54.2
7	Hard Red Winter	10.86	9.19	50.4
24	Hard Red Spring	11.01	9.18	51.4
30	Hard Red Winter Minnturki	11.05	9.27	49.5
23	Hard Red Spring	11.18	9.51	50.1
31	Hard Red Winter "2614"	12.08	10.10	46.1
14	Hard Red Spring Marquis	12.29	10.25	45.4
16	Hard Red Spring	12.39	10.50	44.8
8	White-Baart	12.52	10.37	47.9
20	Hard Red Spring	12.88	10.96	43.7
12	Hard Red Spring Sturgeon	12.90	10.77	42.9
17	Hard Red Spring	13.00	11.14	42.1
11	Hard Red Spring Progress	13.34	11.09	41.7
13	Hard Red Spring Thatcher	13.55	11.38	40.7
18	Hard Red Spring	13.94	11.92	39.4
9	Hard Red Spring	14.39	12.36	38.6
25	Hard Red Spring	14.41	12.42	37.9
27	White	14.68	12.53	38.2
21	Hard Red Spring	14.82	12.83	37.1
22	Hard Red Spring	16.00	13.78	34.7
15	Hard Red Spring	16.26	14.22	33.9

From the curve in Figure 1 it is evident that the protein content can be predicted rather accurately from the light transmission values. Only three of the 34 flour samples show deviations from this relationship of more than 0.3% in terms of protein content, the mean deviation being 0.15% protein.

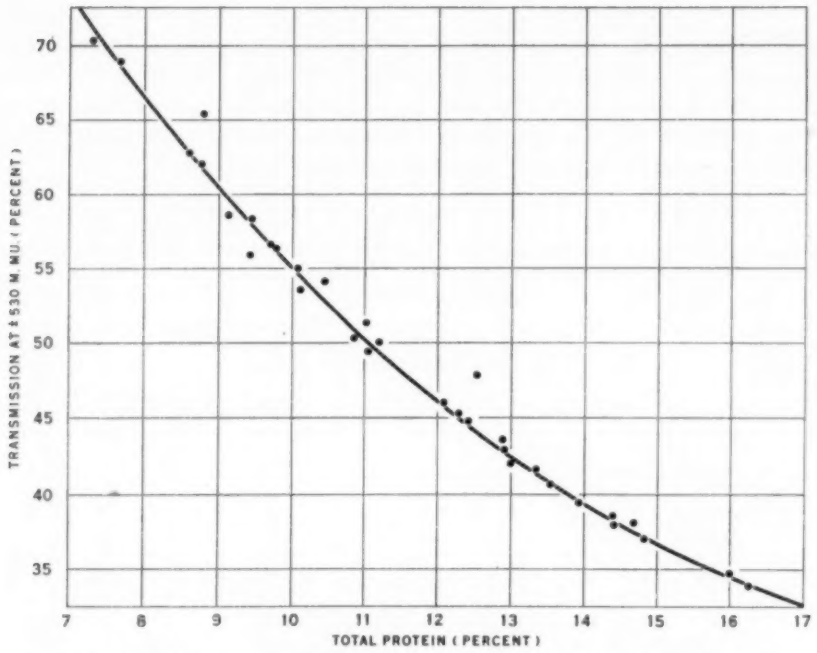

Fig. 1. Relationship between total protein content and light transmission of protein suspension for 34 samples of wheat flour.

Since the turbidity produced in these suspensions is caused by the gluten proteins only, while the albumin and globulin remain completely dispersed, any variation in the normal ratio of gluten to non-gluten protein will cause a deviation in the relationship between total protein content and light absorption. Theoretically, therefore, the light absorption values should be an even better index of gluten protein content than of total protein content.

To test this theory the protein peptizable in 5% potassium sulfate solution was determined on each of the 34 samples of flour under investigation, and by difference the protein not peptizable by the same salt solution was determined. This latter protein fraction may be considered an approximate measure of the gluten protein content, since the gluten proteins are not appreciably dispersed by 5% potassium sulfate. These values also are given in Table II and are shown graphically in Figure 2.

A comparison of Figures 1 and 2 shows a better relationship between light transmission and protein not peptizable with 5% potassium sulfate than between light transmission and total protein. The mean deviation was reduced to 0.13% protein and only one of the 34 samples deviated by more than 0.3% protein from the general relationship as

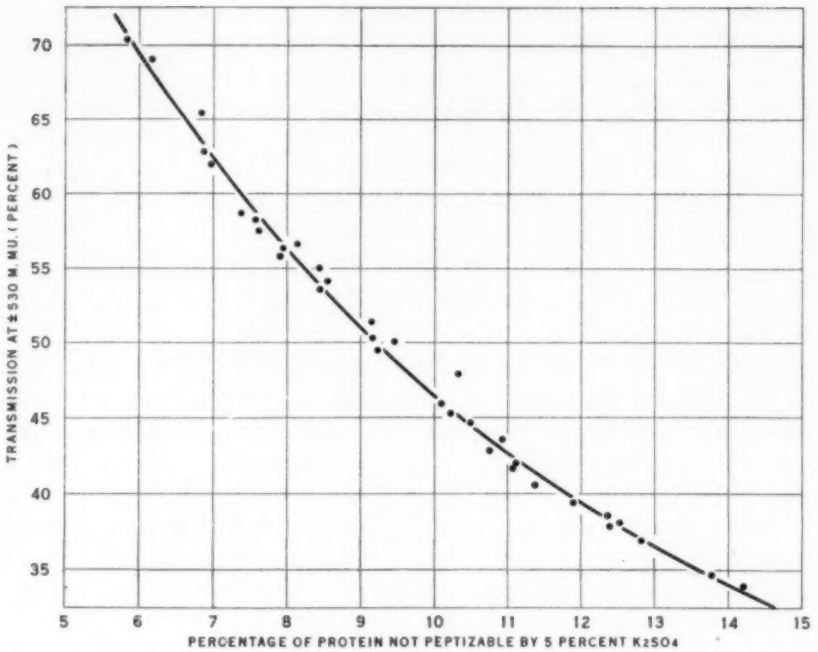

Fig. 2. Relationship between content of protein not peptizable by 5% K_2SO_4 and light transmission of protein suspension for 34 samples of wheat flour.

shown by the curve in Figure 2. It therefore appears that the photoelectric method is a slightly more exact measure of gluten protein content than of total protein content of wheat flour, and should therefore be a slightly better index of baking quality than the total protein content as determined by the Kjeldahl method.

A protein determination by the photometric method as described may be made in about the same length of time as by the Kjeldahl method, and may be made in considerably less time with but little sacrifice in accuracy by allowing only 15 minutes for the development of turbidity. For routine work the photometric method should prove considerably more rapid and less fatiguing to the analyst than the Kjeldahl method.

No table for converting photometric readings into protein percentages is presented herewith, since there is some question as to the uniformity of different makes of photometers, and since the results herein reported were obtained by the use of selected test tubes in the photometer instead of precision absorption cells. The use of such test tubes when well matched is highly desirable for routine work, and the results obtained are of the same order of accuracy as when precision cells are used. However, a conversion table based on such a

series of test tubes cannot be expected to apply to any other series of matched tubes. For the present, therefore, it is recommended that in the application of this photometric method each instrument be calibrated against the standard Kjeldahl procedure for a series of flour samples.

Conclusions

A photometric method for determining the protein content of wheat flour has been developed. The results are in good agreement with those obtained by the standard Kjeldahl procedure and appear to be a somewhat better measure of gluten protein than of total protein content.

The principal advantages of the photometric method for routine work should be the ease and rapidity with which a large volume of work can be handled with a minimum amount of equipment, and without the unpleasant features usually associated with a protein laboratory.

Efforts will be made to adapt the procedure to the determination of protein in wheat as well as flour. The success of this effort would greatly enhance the practical value of the method.

FACTORS THE BAKER SHOULD CONSIDER IN PREPARING THE YELLOW SPONGE CAKE

W. E. Pyke

Colorado Experiment Station, Home Economics Section, Ft. Collins, Colorado

(Read at the Annual Meeting, May 1940)

The Altitude Laboratory of the Home Economics Section of the Colorado Experiment Station has continued a study of the baking of flour mixtures at various altitudes since 1926. In a recent bulletin W. E. Pyke and Gestur Johnson (1940) present certain data regarding preparing and baking yellow sponge cakes at different altitudes. One of the objectives of this study was to establish methods which would permit the true sponge cake to be prepared by means of large commercial mixing equipment. It seemed desirable also to improve, if possible, the general quality of sponge cake by a modification or improvement of methods of mixing used and by the adoption of a dependable formula with a large margin of safety so that the effect of small errors in measurement would be minimized. We believe that these objectives have been accomplished.

No attempt will be made in the present discussion to review the literature. Interested parties are referred to Barmore (1936) and to Pyke and Johnson (1940) for this information.

The commercial baker has used the true sponge cake or "full cream sponge" as it is sometimes called only to a rather small extent, because with the use of large mixing equipment it was difficult to produce it with satisfactory quality. At present prices for materials the cost of the ingredients for a true sponge cake of a size suitable for retail at 35 cents should not exceed 9 cents. This does not include cost of decoration, labor, packaging, and distribution. This relationship should be attractive, provided cakes of high quality can be reproduced with ease with present equipment.

From the beginning of this investigation it became apparent that when large mixing equipment was used the conventional method of mixing the true sponge cake could not be entirely adapted with satisfaction to the production of this type of cake. This was found to be due to the lack of stability of the mixed meringue as prepared by the

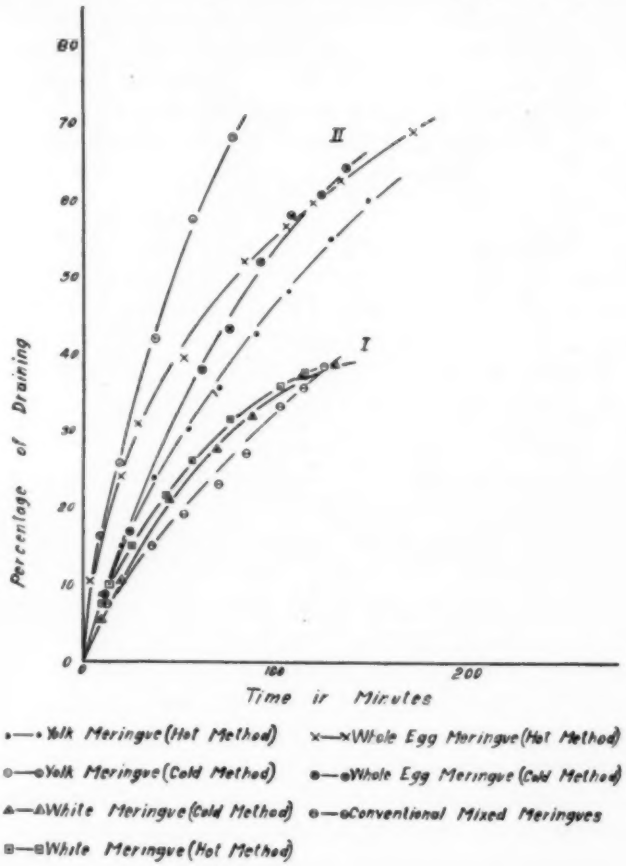

Fig. 1. Percentages of draining of meringues plotted against time.

conventional method and to the inability of the conventional sponge-cake batter to stand the required amount of mechanical manipulation or abuse. Because of this situation a study was made of the properties of various meringues containing egg yolk. These properties were related to the properties of sponge-cake batters which could be prepared therefrom by various methods of mixing. Figures 1 and 2 show some of these properties and relationships.

In Figure 1 the percentage of draining of a 36-g. sample of each completed meringue through filter paper is plotted against time. For convenience of consideration these curves have been divided into two

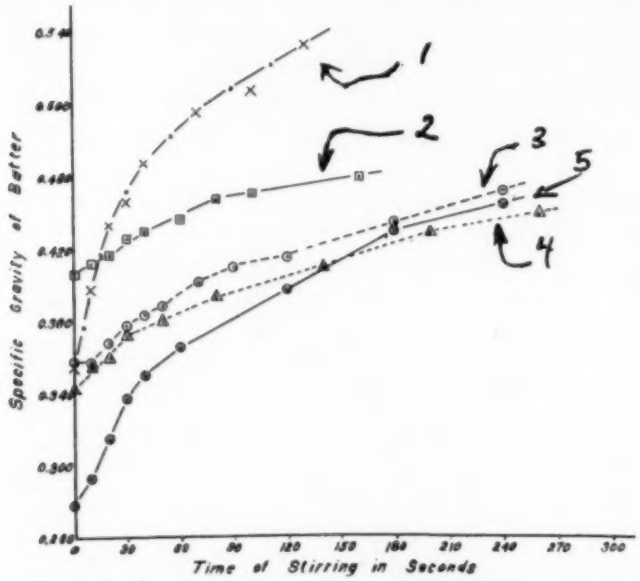

Fig. 2. Effect of stirring at slow speed by means of the commercial-type mixer upon sponge-cake batters prepared by different methods of mixing. Curve 1, conventional method of mixing, hand folding. Curve 2, hot single meringue method, flour added while meringue was hot. Curve 3, cold single meringue method of mixing. Curve 4, hot single meringue method, flour added after cooling meringue below 30°C. Curve 5, meringue beaten by cold single method, flour folded in by hand to point of origin, mechanical manipulation thereafter same as that of other batters.

groups. Group I includes the more stable slow-draining meringues and Group II the less stable rapid-draining meringues. Briefly, the method of preparation of these meringues was as follows. The sugar, salt, and cream of tartar were practically in solution in the egg magma before whipping was started. The egg substance and water used, when water was an ingredient, had been mixed by beating a few seconds at high speed before the solids were added. The cold-method meringues were prepared by whipping the mixture at high speed at room temperature with the commercial-type mixer. The hot-method meringues were prepared similarly except that the three-quart bowl of the

mixer was immersed in a hot water bath at 80°C. during the beating. After beating, the bowl containing the hot meringue was immersed in cold water and the meringue was cooled quickly to 30°C., except in the case where the characteristics of the meringue while hot were being investigated (Curve 2, Fig. 2).

The composition of the meringue samples for the draining experiments and for the preparation of the corresponding batters follows. Egg-white meringues contained 150 g. sugar, 1 g. salt, and 4 g. cream of tartar to 210 g. egg white. Whole-egg meringues contained 134 g. sugar, 1 g. salt, 1 g. cream of tartar, and 30 cc. water to 192 g. whole egg (120 g. white and 72 g. yolk). Each yolk meringue contained 150 g. yolk, 130 g. sugar, 75 cc. water, and 1 g. each of salt and cream of tartar. For the conventional meringue one-half the sugar and the cream of tartar were whipped up with the white, and one-half the sugar and the water were beaten with the yolk. The resulting meringues were folded together either by machine or by hand as the purpose of the investigation demanded.

When these meringues were converted into cake batters, the following procedures were used. In the conventional method the flour was folded into the meringue during 3½ minutes either by hand or mechanically. Mechanical folding in the formation of conventional sponge-cake batter produced a batter of too high specific gravity for the production of a satisfactory true sponge cake at low altitudes. For all other batters the flour was added in 6½ minutes with continued stirring at slow speed by the commercial-type mixer. As a test of stability of the resulting batters, the stirring of these batters was continued in the commercial-type mixer at slow speed after the batter had been completed. At intervals the machine was stopped and the specific gravity of the batter determined. The batter used for the determination was returned to the mixing bowl and the stirring continued. The batter obtained from the yolk meringue was found to be too stable to obtain any appreciable effect on its lightness by this type of mechanical manipulation. For this reason there is no comparison of this type of batter in Figure 2.

It is apparent from a study of the curves represented in these two figures that the rapid-draining meringues produced far more stable batters. The batters produced by this improved method of mixing are sufficiently stable for production on the large scale with the use of heavy equipment. They are easily reproducible and yield low-cost cakes of excellent quality. That their texture is far superior to that of the conventional-type sponge cake is indicated in Figure 3. Other quality measurements also showed them superior.

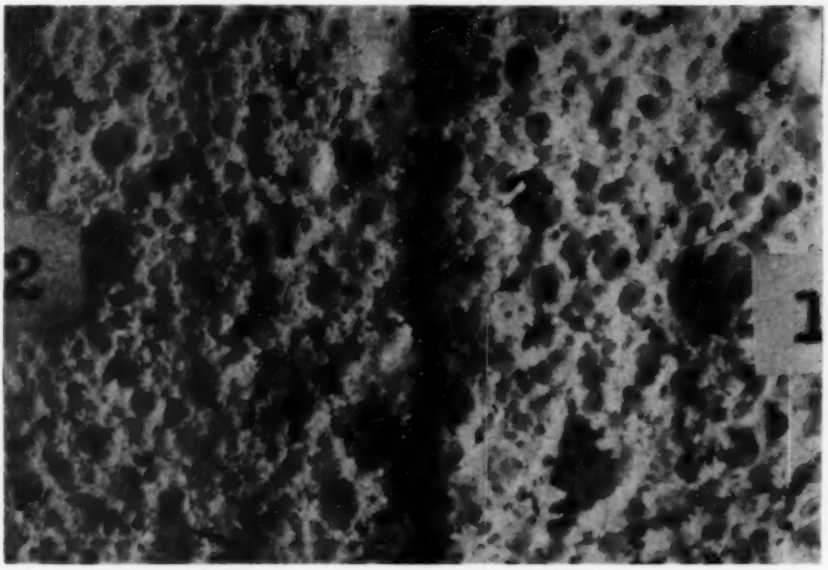

Fig. 3. Sample 1 was prepared by the conventional method of mixing sponge cakes. Sample 2 was prepared by the improved single-meringue method. Magnification three diameters.

The basic sponge-cake formula used for 5,000 feet altitude was:

Egg white	120 g.	Cream of tartar	1 g.
Egg yolk	72 g.	Salt	1 g.
Cake flour	77 g.	Flavoring	2 cc.
Sugar	134 g.	Water	30 cc.

This formula produced a cake of medium size. The sugar content was about the average of available sea-level recipes. A study (Pyke and Johnson, 1940) indicated that a cake with a tensile strength of about 17 g. per square centimeter could be obtained at 5,000 feet altitude through the use of this formula when the leavening was correct and proper techniques were followed.

Evidence supporting the statement that these improved methods of mixing are superior to the conventional method is shown in Figure 4. Throughout this figure the crosses represent cakes produced from conventional sponge-cake batters, the circled crosses represent cakes from a transition-type batter, and the circles represent cakes from batters mixed by the improved single-meringue method. The transition-type batters were started like the conventional batter. The white and yolk meringues were then mixed and beaten together at high speed. The mixed meringues at first lost volume and then rapidly regained it. The flour was added over 6½ minutes at low speed as with the improved single-meringue method. Each cake was obtained from 380 g. of batter. Baking conditions were controlled and were

the same throughout. All these cakes were mixed through all steps of preparation with the commercial-type mixer. It is evident that the improved single-meringue method of mixing is to be preferred when large mechanical equipment is to be used.

It has been common practice to omit measurement upon one of the ingredients of the true sponge cake. This ingredient is the leavening

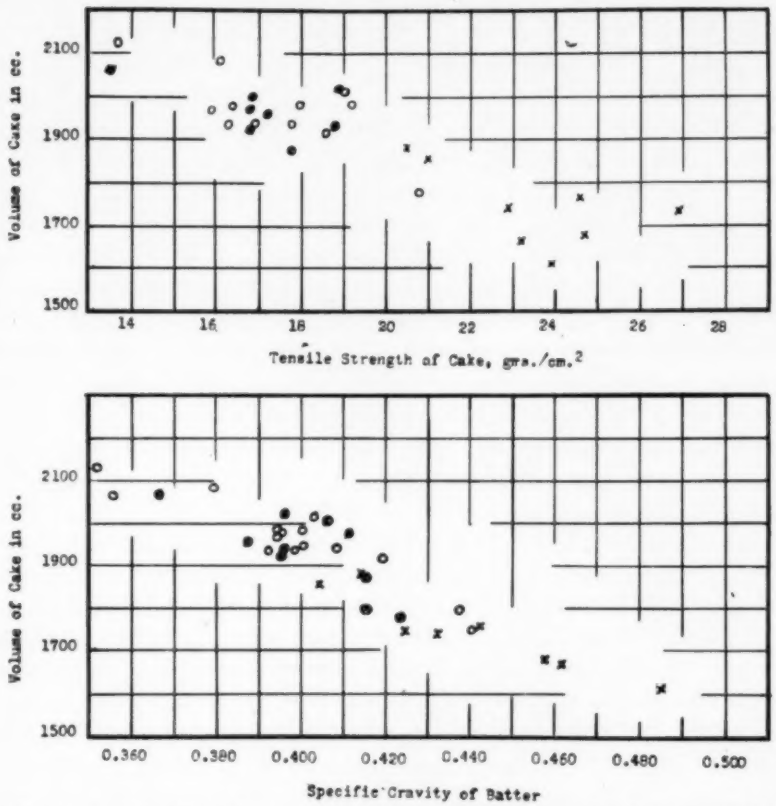

Fig. 4. A comparison of three methods of mixing the true sponge cake when commercial-type mixing equipment is used throughout. The crosses represent batters and cakes prepared by the conventional method. Circles represent batters and cakes prepared by the cold single meringue method. Filled circles represent a transition-type batter. Lack of stability of the conventional-type batter on the machine caused the production of a heavy batter of little practical value. The other methods are suitable for machine use.

air. It is apparent that such an omission could prove ruinous if large-scale production were attempted. Without control of the leavening a standardized product is impossible. With this control, good technique, and standard equipment, a highly attractive, readily reproducible product is possible. The leavening agent in the true sponge cake can be measured indirectly by the determination of the specific gravity or specific volume of the batter. Figure 5 shows the relationship between

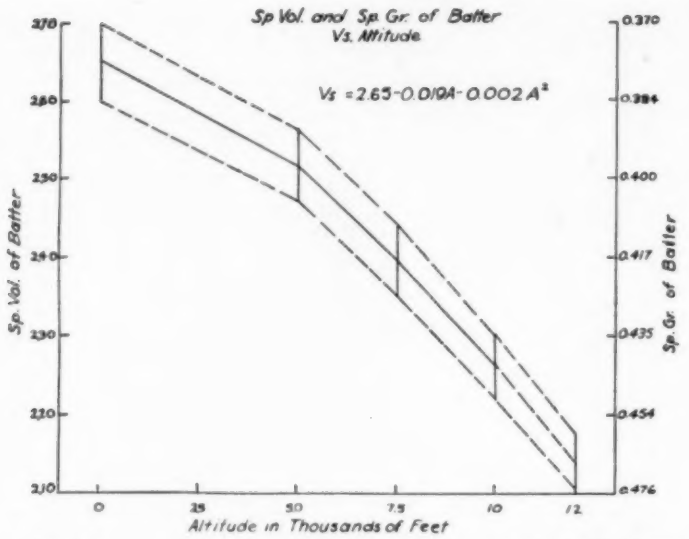

Fig. 5. As altitude increases the specific gravity of the sponge-cake batter should increase if cakes of comparable volume and texture are to be obtained.

these values for proper leavening effect at different altitudes. This curve applies directly to the cake formulas to be given later. It will apply to other true sponge-cake formulas, but the intercepts may be altered in such cases.

Certain other factors are very important when large equipment is to be used in mixing the true sponge cake. Fisher (1936) has called attention to the interrelationships that exist between size and type of mixing equipment and the results obtained with the A.A.C.C. baking method. In the preparation of sponge-cake batters the interrelationship of size of bowl and whip to the size of the batch and speed is most important. Underloading or overloading a bowl of any particular size may lead to costly and disappointing results. The effective whip speed is definitely related to the size of the bowl. Within limits as the size of bowl and batch increase, the speed of the whip should decrease. If, then, bowls of varying sizes are to be used on the same machine, provision for a multiplicity of speeds should be available.

The bakery engineer has made steady improvement of available equipment and is to be commended on the remarkable progress that has been made. In spite of this, however, the available speeds upon a machine may not suit the case in hand. When two speed ratios are available, one of which is too violent to complete the meringue to its desired lightness, and the other too slow, our experience has shown that a meringue of the desired lightness can be formed by whipping with the higher speed until its maximum effect has been obtained and then

shifting to the next lower speed. This takes longer than the correct speed ratio, but it works. The important thing is to get the whole-egg or yolk meringue light enough. A specific gravity of 0.22 for the meringue at 20° to 30°C. is safe. A lighter meringue will not be detrimental.

Bakers for a long time have depended upon some fixed time interval for the control of mixing processes. While this is safe with butter-type cakes, its safety is doubtful with the sponge cake, both the angel food and yellow sponge. Eggs of different age and quality form meringues at very different rates. If time is the criterion used to determine when the meringue is completed, the cake batters formed from these meringues will have quite different characteristics. That the cakes from such batters vary considerably is evidenced by the observations of numerous investigators (Barmore, 1936; Hunt and St. John 1931; Glabau, 1940). Barmore (1936) has shown that if the egg-white foams are beaten to the same specific gravity identical cakes result. An implied proviso to this statement is that the egg whites should not vary too greatly in quality. This qualification arises from the fact that egg-white foams of the same specific gravity but varying considerably in quality shrink differently when the flour is added. The shrink in the oven and after baking also is quite different; so eggs of average or higher quality should be used. With the yellow sponge cake, prepared by the improved method we have outlined, wide and notable differences in rate of meringue formation with eggs from different grades need not affect the lightness of the finished batter, if the procedure outlined is followed. Specific gravity determinations and not time should be used to indicate when the batter is completed, if high quality, uniformity, and reasonable economy are to follow.

Another relationship with which the baker is familiar is of considerable importance in the baking of the yellow sponge cake. This is pan type, size, and shape. Their relationship to the quantity and lightness of the batter is apparent. Since sponge-type cakes are supported considerably during cooling by their grip upon the surface of the pan, the span between surfaces should not be too great, or contraction will produce a dip midway across the span. Related factors here are the elasticity and tensile strength developed within the cake during baking. Within the temperature range used, elasticity and tensile strength for any given formula are directly proportional to the internal temperature attained during baking. This approximates 100°C. at sea level and drops about 1°C. for each 1,000 feet above sea level in altitude. Near the hot pan surface this temperature is considerably exceeded. The greatest strength of the structure is developed adjacent to the pan surface, provided the sugar-water ratio has been correct for

the pressure conditions under which the cake was baked. If the sugar-water ratio has been too high, a sticky semisolid layer arises next to the pan. As the cake cools, this sticky layer fails to hold. Various stages of collapse due to contraction may result from such conditions. The greater portion of the water evaporation occurs in this highly heated layer. Sugar solution diffuses into this layer to replace continuously the water evaporating. If the relative sugar concentration becomes too great in this layer, neither starch nor egg substance sets properly and a sticky layer results.

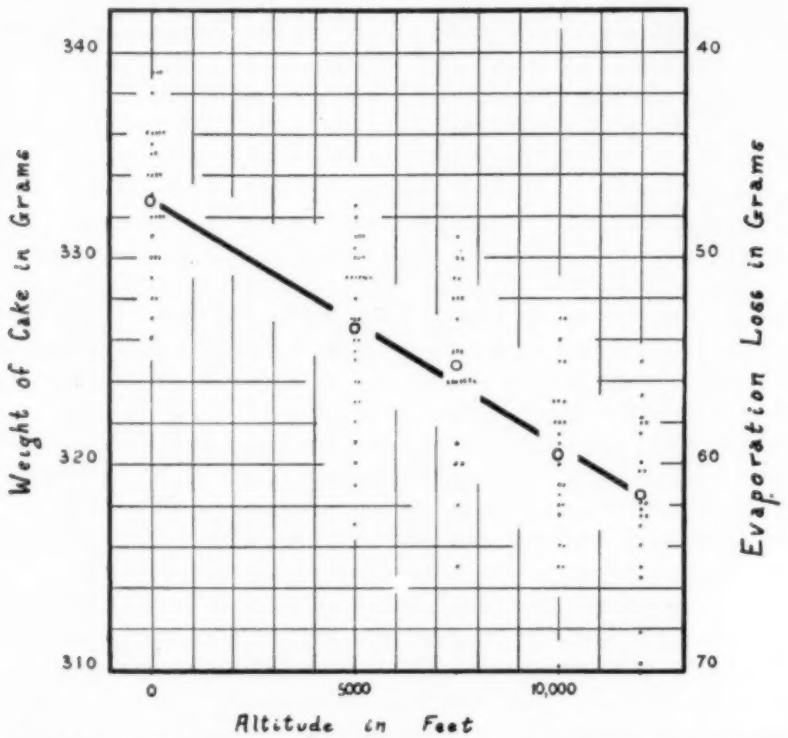

Fig. 6. Weight losses when 380 g. of batter are baked at different altitudes. The water content of sponge-cake batters should be increased as the altitude increases, to correct for increased evaporation.

Since the rate of evaporation increases as the atmospheric pressure decreases, more water per gram of sugar must be present at high altitudes than at low altitudes. Figure 6 indicates how this rate of evaporation is changed as the altitude increases. To provide a reasonable margin of safety, the water correction for the cake formula should exceed slightly the amount indicated. When the water corrections indicated in the sponge-cake formulas given later are made, the sugar solution within the cake will vary between 52% and 54% sugar over

the altitude range from 0 to 12,000 feet, if we assume uniform distribution of the sugar in the water. These variations arise largely from practical considerations in adjusting the recommended measurements to reasonably convenient values. It is possible to balance sponge-cake batters higher in sugar content which may be used successfully over this altitude range, but organoleptic tests upon cakes from such batters did not indicate any appreciable increase in the sweetness of the product to the taste. By other quality measures these cakes still higher in sugar content were not superior but were inferior to the cakes with the sugar content chosen. Economy considerations also favor the sugar content selected. In Figure 7 we see the results one may expect to

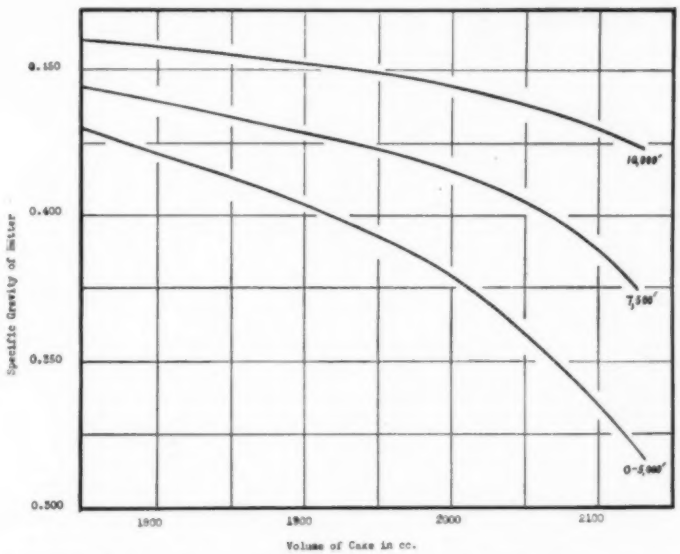

Fig. 7. A nomograph showing the volume expected from 380-g. samples of sponge-cake batter of different specific gravities when baked at different altitudes.

obtain with the use of the whole-egg sponge cake formula at different altitudes, when 380 g. of batter having different specific gravities are used. The standard deviations of the volumes of cakes for a variation of 0.01 in specific gravity in the 1,950-cc. range at 5,000 feet altitude was found to be 40 cc. in a series of 14 cakes. The error included the effects of the natural fluctuations in atmospheric pressure at 5,000 feet as well as other sources of error. The standard deviations in the 2,050-cc. range ran slightly lower. We found no difficulty from a practical standpoint in controlling the specific gravity to a variation of 0.003. It is evident from Figure 7 that a more precise control of the specific gravity of the batter should occur at high altitudes than at low altitudes to obtain the same uniformity in volume.

A study of the effect of altitude upon the tensile strength of sponge cakes is shown in Figure 8. The procedure used in arriving at these values is described elsewhere (Pyke and Johnson, 1940). The equation given, $T = 19.9 - 0.03A - 0.114A^2$, applies to cakes of a specific gravity of 0.168. It will be noted that these sponge-cake formulas yield more tender sponge cakes than have heretofore been recommended. They are of much higher quality and have better keeping

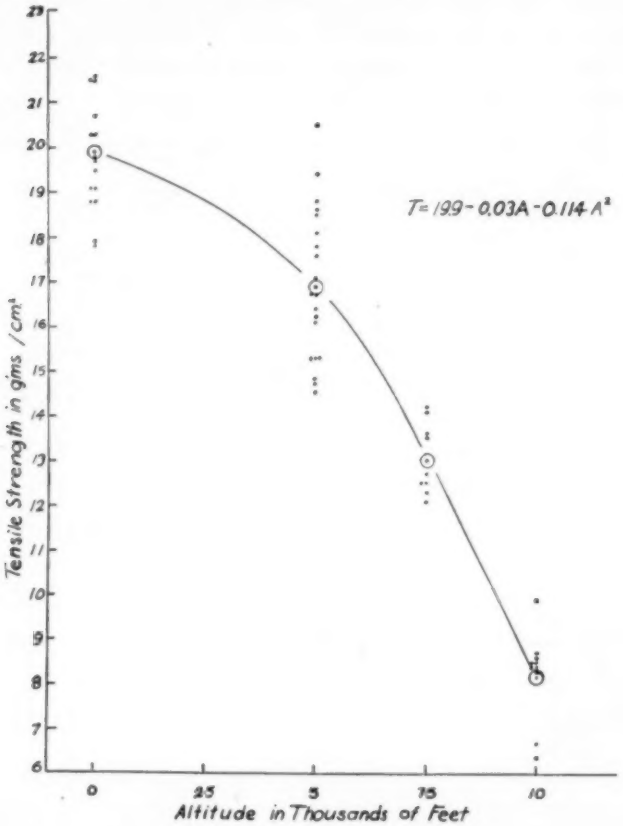

Fig. 8. Relation of tensile strength to altitude.

characteristics than those of heavier, tougher cakes. The improved method of mixing described produces a texture (Fig. 3) superior to the conventional method so that taste impressions are improved, probably because of the accompanying more velvety, more moist sensation on the tongue.

The formulas and procedures for yellow sponge cakes are taken from the Colorado Experiment Station Technical Bulletin 27 (Pyke and Johnson, 1940).

WHOLE-EGG SPONGE CAKE FORMULA

Ingredients	Altitude	Metric formula	Commercial formula		Housewife's formula (volume measure)		
	Ft.	*Grams*	*Lbs.*	*Oz.*	*Cups*	*T.*	*t.*
Cake flour sifted once before measuring	0 to 5,000	77	2	0	¾+	1+	2
	7,500	85	2	4	1−	1−	2
	10,000	95	2	8	1+	1+	0
	12,000	105	2	12	1+	2+	2
Water[1]	0	20	0	8	—	1+	1
	2,500	25	0	10	—	1+	2
	5,000	30	0	12½	—	2+	0
	7,500	30	0	12½	—	2+	0
	10,000	35	0	14	—	2+	1
	12,000	40	1	0	—	2+	2
Egg white	Any	120	5 pounds whole eggs		4 medium-sized eggs		
Egg yolk	Any	72					
Sugar	Any	134	3	8	⅔+	0+	0
Salt	Any	1	0	½	0+	0+	¼
Cream of tartar	Any	1	0	½	0+	0+	¼
Vanilla [1]	Any	2 cc.	0	1	0+	0+	½
			Grams		*Ounces*		
Correct weight of 1 cup (net) of batter for proper leavening	0		87 to 91		3 to 3⅛		
	2,500		90 to 93		3⅛ to 3¼		
	5,000		92 to 96		3¼ to 3⅜		
	7,500		97 to 100		3 7/16 to 3½		
	10,000		103 to 106		3⅝ to 3¾		
	12,000		109 to 112		3 13/16 to 3 15/16		

[1] It is often desirable to include lemon juice and grated lemon peel as a flavor in sponge cake in addition to the small amount of vanilla advised. The amount of water to be added should be corrected for the amount of lemon juice to be added. Five cc. or 1 teaspoon of juice and 1 tablespoon of freshly grated peel should be added at the end of the mixing period for the metric and housewife's formula. For the commercial formula 2¼ ounces of juice and 1 ounce of grated peel should be used.

Procedures

A. Single Meringue Method of Mixing

Cold method.—Equipment recommended for this method of mixing is an efficient mechanical mixer with a wire whip. The egg whites, yolk, and water required are cut together with the beater to mix them thoroughly. The sugar, salt, cream of tartar, and vanilla are dissolved in the egg, and the mixture is beaten until the meringue is very light and just fails to stand in a peak. The mixer is changed to slow speed and the cake flour added slowly over a period of six minutes. The bowl is thoroughly scraped down, the lemon juice and grated peel added if desired, and mixing continued one-half minute at slow speed. The batter is then weighed in a standard tared cup. If necessary (this is always the case at high altitudes), stirring is continued at slow speed until a cup of batter gives the required net weight. Baking should be

in an unlined, ungreased tube pan with removable bottom. Cakes should be inverted to cool. For jelly roll the heavier of the recommended weights should be used for each altitude. The jelly roll batter should be baked in a large, flat, lined pan.

Hot method.—The hot method is recommended for hand equipment with double Dover beater, small mixing equipment, and large commercial equipment. This method is the same as the cold method except that the bowl containing the egg mixture after the solids have dissolved is placed in a pan of hot water (80°C. or 175°F. for the greatest effect) during the beating period. The meringue comes up much more rapidly by the hot method. When the meringue forms a soft peak, the bowl is placed in cold water and the contents cooled with occasional stirring. The cake flour is added as directed under the cold method. The batter gives a larger volume by the hot method than by the cold method. The heavier of the net weights for a cup of batter is to be preferred for each altitude.

B. Conventional Method of Mixing

Equipment recommended is either hand equipment or a combination of hand and mechanical equipment. The whites and yolks are beaten up separately. The water required, the salt, and the vanilla are added to the yolks and mixed by a few seconds of beating. One-half of the sugar required for the formula is added and stirred until mostly dissolved. This mixture is beaten until it forms a soft-peaked meringue. The whites with the cream of tartar and one-half the sugar are beaten to a meringue that just peaks but is not too stiff. The two meringues are folded together. The flour is then folded into the mixed meringue. If lemon juice and peel are desired they are also folded in. Folding and mixing the batter are continued until the required leavening density is reached as determined by the weight of a cup of batter. This method of mixing does not give as desirable a cake as is obtainable by procedure *A*. The mixed meringues and the finished batter are very sensitive to mechanical abuse. Baking directions are the same as for the cake mixed by the single meringue method.

BAKING TIME AND TEMPERATURES

Altitude	Degrees C.	Degrees F.	Time in minutes [1]
0 and 2,500	165	330	50–55
5,000 and 7,500	171	340	55–60
10,000 and 12,000	177	350	63–65

[1] Baking time depends upon shape of pan and its size. The time recommended is for a batter of 380 g. or 13½ oz. (the 4-egg cake) in a pan permitting the height of the cake to approximate the distance from the tube to the side of the pan, measured at the top of the cake. A flat sheet for jelly roll or smaller batches should be baked for shorter periods of time. In a controlled oven, the top heat should be low and the bottom heat high.

YOLK SPONGE CAKE FORMULA[1]

Ingredients	Altitude	Metric formula	Commercial formula		Housewife's formula (volume measure)		
	Ft.	*Grams*	*Lbs.*	*Oz.*	*Cups*	*T.*	*t.*
Cake flour sifted once before measuring	0 to 5,000	100	2	0	1+	2+	0
	7,500	110	2	3	1¼+	0+	2
	10,000	130	2	6	1½−	0−	2
	12,000	130	2	9	1½+	2+	0
Water	0	70	1	6	¼+	0+	2
	2,500	75	1	8	¼+	1+	0
	5,000	85	1	11	¼+	1+	2
	7,500	90	1	12⅔	¼+	2+	0
	10,000	100	2	0	¼+	2+	2
	12,000	110	2	3	¼+	3+	1
Egg yolk	Any	150	3	0	8 egg yolks		
Sugar	Any	134	2	11	⅔+	0+	0
Salt	Any	1	0	½	0+	0+	¼
Cream of tartar	Any	1	0	⅕	0+	0+	¼
Vanilla	Any	2 cc.	0	1	0+	0+	½
			Grams		*Ounces*		
Correct weights of 1 cup (net) of batter for proper leavening	0		91 to 93		3⅛ to 3¼		
	2,500		93 to 95		3¼ to 3⅜		
	5,000		98 to 100		3 7/16 to 3½		
	7,500		103 to 106		3⅝ to 3¾		
	10,000		112 to 115		3 13/16 to 4 1/16		
	12,000		118 to 120		4⅛ to 4¼		

[1] This cake is far more tender than the usual sponge cake. It requires more careful handling, but its high quality recommends it. The baking temperature for the yolk sponge cake is the same as for the whole-egg sponge cake. The baking time should be increased by 10 minutes. *Top heat should not be used.*

Summary

New methods of mixing have been investigated and described. They have been adapted for use with large commercial equipment as well as with small equipment. These new methods produce sponge cakes which are superior to the conventional-type sponge cake.

Factors which will concern the commercial baker when he produces the true sponge cake have been discussed. If the recommendations are followed, it should be possible to produce high-quality, low-cost cakes of the true sponge type upon a large scale.

The leavening of true sponge cakes (angel food and yellow sponge) should be controlled by specific gravity determinations upon the batter. Time of beating as a factor to control this ingredient is not dependable.

Formulas for the preparation of yellow sponge cakes at various altitudes have been given. Interpolation between the intervals of 2,500 feet is permissible.

The formulas for yolk sponge cakes adjusted to various altitude conditions yield cakes which compare to butter cakes in tenderness. Their quality is high. They require a longer baking period than the whole-egg sponge.

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A PRELIMINARY SURVEY OF THE VITAMIN B_1 CONTENT OF AMERICAN CEREALS

ALFRED S. SCHULTZ, LAWRENCE ATKIN, and CHARLES N. FREY

The Fleischmann Laboratories, Standard Brands Incorporated, New York, N. Y.

(Read at the Annual Meeting, May 1940)

Of all the vitamins known to man, vitamin B_1 appears to be most closely related to carbohydrate metabolism. Also, of all the foods known to man, the cereals are the chief source of carbohydrates. Furthermore, the carbohydrates provide the major part of the calorie intake of the average human diet. It is a generally accepted theory that the utilization of each gram of carbohydrate requires a specific amount of vitamin B_1.

The determination of vitamin B_1 in cereals has heretofore been somewhat difficult because of the relatively low B_1 content. The fermentation method for B_1 determinations developed at our laboratories [1] is, however, very convenient for such measurements. With this method we have analyzed some 130 grains in a preliminary survey designed to determine the average B_1 content, the range of values, and the influence of variety.

The method of analysis has already been published, and in the present communication only the preparation of the sample will be described. The seed was ground in a Seck mill at the finest setting.

[1] A. S. Schultz, L. Atkin and C. N. Frey. A fermentation test for vitamin B. J. Am. Chem. Soc. **59**: 2457–2460, 1937. The vitamin B_1 content of wheat flour and bread. Cereal Chem. **16**: 643–648, 1939.

One to two grams of the meal were weighed into a 100-ml. volumetric flask and about 50 ml. of water added. Sufficient sulfuric acid was added to make the reaction blue to congo red paper and then it was heated in flowing steam for 20 minutes. When cool, the solution was neutralized to neutral litmus with NaOH and made up to volume. Aliquot fractions of the well stirred suspension were transferred to the reaction or fermentation bottles by a fast-flowing pipette. It is sometimes more convenient to take aliquots with a graduated cylinder. The reproducibility of the technique is reasonably high; checks do not usually differ by more than 5%.

The data which we have obtained cannot be considered as being absolutely representative of the various grains. Too few samples have been examined as yet. The wheats, for example, comprise a random group, including durum, and the results are no more representative of soft than of hard wheats. However, the results of this preliminary survey may be of interest in view of the observed variability in the vitamin content, which shows the necessity for further research.

Results

Thirty-one wheat samples in all were assayed.[2] The data are given in Table I. The values ranged from 4.2 to 7.3 γ per gram. The average thiamin content is 5.6 γ per gram. This average is of doubtful significance since the samples make a heterogeneous group. Insufficient data have been gathered, as yet, to draw any conclusions regarding the exact effect of climate, soil, etc., but since there are significant differences throughout the group one may assume that these factors are not without effect.

The assays of 37 barleys are given in Table II. With a range of 3.8 to 9.2 they averaged 6.2 γ thiamin per gram. The great spread in values is evidenced by the fact that eight samples were higher than 7.2.

In the case of the barleys we were fortunate in having a certain amount of data on grades and strains. Table III shows how the thiamin content of barley appears to vary with the grade. The No. 1 grade which has the largest kernel size has the lowest average thiamin content. These differences are probably related to the distribution of thiamin between the various parts of the seed; *i.e.*, the No. 1 grade may have a larger proportion of a low thiamin-bearing constituent.

Table IV shows that three samples of Oderbrucker averaged 6.17 against 5.55 γ thiamin per gram for four samples of Wisconsin 38.

[2] We would like to thank the following men and organizations who so kindly supplied us with the samples of grain: Dr. C. H. Bailey, University of Minnesota, St. Paul, Minn., Dr. H. W. Brown, Michigan State College, East Lansing, Mich., Dr. S. Jozsa, Fleischmann Malting Co., Chicago, Ill., Malt Research Institute, Madison, Wis., Dr. H. S. Smith, U. S. Dept. of Agriculture, Washington, D. C., Dr. A. F. Swanson, U. S. Dept. of Agriculture, Hays, Kan., Dr. H. L. Westover, U. S. Dept. of Agriculture, Washington, D. C., Dr. E. B. Working, Kansas State College, Manhattan, Kan.

TABLE I

WHEAT ASSAYS

Lab. No.	Name	Obtained from	Thiamin, γ per gram
9F175 A	Spring—Mindum Durum	St. Paul, Minn.	7.1
9F175 B	Spring—Thatcher	" " "	7.3
9F175 C	Winter—Minturki	" " "	6.9
9F208 A	Early Blackhull C.I. No. 8856	Manhattan, Kan.	4.5
9F208 B	Kanred C.I. No. 5146	" "	5.0
9F208 C	Blackhull C.I. No. 6251	" "	5.2
9F208 D	Turkey C.I. No. 1558	" "	4.5
9F208 E	Tenmarg C.I. No. 6936	" "	5.7
9F208 F	Kawvale C.I. No. 8180(8186?)	" "	4.5
9F208 G	Clarkan C.I. No. 8858	" "	6.7
9F208 H	Chiefkan C.I. No. 11754	" "	4.7
9F208 I	Nebred C.I. No. 10094	" "	6.0
9F208 J	Cheyenne C.I. No. 8885	" "	5.2
9F208 K	Kharkof C.I. No. 1442	" "	4.5
9F208 L	Oro X Tenmarg C.I. No. 11673	" "	6.0
9F088 A	Spring 13.5% prot.	Chicago, Ill.	5.9
9F088 B	White 14.4% prot.	" "	6.1
9F088 C	No. 1 Amber Durum 11.9% prot.	" "	5.9
9F088 D	No. 1 Hard Winter 14.5% prot.	" "	6.7
9F088 E	No. 1 Hard White 13.5% prot.	" "	6.1
9F088 F	White	" "	6.1
9F088 G	Minnesota Winter 13.5% prot.	" "	5.9
9F088 H	Minnesota Winter 11.6% prot.	" "	5.5
9F088 I	Red Durum 14.5% prot.	" "	6.3
9F088 J	Montana Winter 15.0% prot.	" "	6.0
9F088 K	Montana Spring	" "	5.8
0F144 A	Nittany	East Lansing, Mich.	5.2
0F144 B	Baldrock	" " "	4.2
0F144 C	American Banner	" " "	4.5
0F144 D	Trumbull	" " "	4.7
0F144 E	Purkoff	" " "	5.1

With regard to the specific effect of any given region little can be said except to conclude that such effects do exist. Further work, which will probably be done by those most interested, should give the answer.

Twenty-three samples of corn (Table V) averaged 5.34 γ per gram. With the exception of one sample at 8.0, all the samples fell between 4.1

TABLE II

BARLEY ASSAYS

Lab. No.	Name	Obtained from	Thiamin, γ per gram
9F137 A	Club Mariout	Hays, Kan.	6.3
9F137 B	Spartan	" "	7.2
9F137 C	Vaughn	" "	7.1
9F137 D	Stavropol	" "	8.7
9F137 E	Flynn	" "	6.5
9F137 F	Trebi	" "	8.2
9F137 G	Atlas X Vaughn	" "	7.7
9F137 H	Atlas	" "	7.5
9F137 I	White Smyrna	" "	7.1
9F137 J	Lico	" "	7.1
9F137 K	Franklin (Oderbrucker)	" "	6.2
9F176 A	Wisconsin No. 38	St. Paul, Minn.	6.5
9F176 B	Velvet	" " "	7.5
9F176 C	Improved Manchuria	" " "	9.2
0F141 A	Spartan	East Lansing, Mich.	5.7
0F141 B	Wisconsin No. 38	" " "	5.5
9F087 A	"D" South Dakota	Chicago, Ill.	6.1
9F087 B	Illinois	" "	6.1
9F087 C	"D" North Dakota	" "	6.1
9F087 D	"D" Wisconsin	" "	5.6
9F087 E	No. 2 North Dakota	" "	5.6
9F087 F	No. 1 Minnesota	" "	5.1
9F087 G	No. 2 Minnesota	" "	5.6
9F087 H	No. 1 Michigan	" "	5.1
9F087 I	No. 2 Michigan	" "	5.3
9F087 J	No. 1 North Dakota	" "	4.7
9F087 K	Red River Valley (N.W. Minnesota)	" "	5.3
9F087 L	Oderbrucker (Wisconsin)	" "	6.6
9F087 M	No. 1 South Dakota	" "	5.8
9F087 N	Pedigree 38 (Wisconsin)	" "	5.5
9F087 O	No. 1 Wisconsin	" "	5.1
9F087 P	No. 2 Wisconsin	" "	5.1
9F087 Q	No. 1 Hanna (2 row) (Oregon)	" "	3.8
9F087 R	No. 2 Hanna (2 row) (Oregon)	" "	4.7
9F087 S	No. 2 Iowa	" "	5.0
9F195	Oderbrucker	Madison, Wis.	5.7
9F196	Wisconsin 38	" "	4.7

TABLE III

RELATION OF BARLEY GRADES TO THIAMIN CONTENT

Sample marked	Grade		
	No. 1	No. 2	D
	γ per g.	γ per g.	γ per g.
Minnesota	5.1	5.6	—
North Dakota	4.7	5.6	—
Michigan	5.1	5.3	—
South Dakota	5.8	—	6.1
Wisconsin	5.1	5.1	5.6
Oregon	3.8	4.7	—
Average	4.93	5.26	5.85

TABLE IV

THIAMIN CONTENT OF TWO BARLEY STRAINS

Sample obtained from	Wisconsin No. 38	Oderbrucker
	γ per g.	γ per g.
Illinois	5.5	6.6
Wisconsin	4.7	5.7
Minnesota	6.5	—
Michigan	5.5	—
Kansas	—	6.2
Average	5.55	6.17

TABLE V

CORN ASSAYS

Lab. No.	Name	Obtained from	Thiamin, γ per gram
9F177 A	Minnesota No. 13	St. Paul, Minn.	5.6
9F177 B	Rustler	" " "	5.8
9F207 A	Colby Yellow Cap (Kan.) C.I. No. 2894	Manhattan, Kan.	5.0
9F207 B	Cassel White (Kan.) C.I. No. 2891	" "	6.1
9F207 C	Commercial White (Kan.) C.I. No. 2881	" "	5.5
9F207 D	Midland (Kan.) C.I. No. 2877	" "	5.1
9F207 E	Hays Golden (Kan.) C.I. No. 2875	" "	6.0
9F207 F	Reid (Kan.) C.I. No. 2873	" "	5.5
9F207 G	Pride of Saline (Kan.) C.I. No. 2867 ?	" "	6.0
9F207 H	Yellow Selection No. 1 C.I. No. 2768	" "	5.3
9F207 I	Iowa 939 (Nebraska) C.I. No. 2887	" "	4.6
9F207 J	US 13 (Ohio) C.I. No. 2885	" "	4.1
9F207 K	US 44 (Illinois) C.I. No. 2876	" "	4.6
0F193 A	Pickett	East Lansing, Mich.	4.1
0F193 B	Duncan	" " "	5.8
0F193 C	M.A.C. Yellow Dent	Washington, D.C.	4.7

TABLE V—(*Continued*)

Lab. No.	Name	Obtained from	Thiamin, γ per gram
0F193 D	Northwestern Dent	Washington, D.C.	5.0
0F194 A	Gehu Yellow Flint (North Dakota)	" "	8.0
0F194 B	Dakota White Flint (North Dakota)	" "	6.0
0F194 C	Golden King (Iowa)	" "	6.0
0F194 D	Krug (Iowa)	" "	4.8
0F194 E	Whatley Prolific (Georgia)	" "	4.5
0F194 F	Garrick Prolific (South Carolina)	" "	4.7

TABLE VI

OAT ASSAYS

Lab. No.	Name	Obtained from	Thiamin, γ per gram
0F174 A	Rusota	St. Paul, Minn.	9.6
9F174 B	Minrus	" " "	10.0
9F174 C	Gopher	" " "	10.3
9F174 D	Anthony	" " "	8.2
9F174 E	Iogold	" " "	9.7
9F206 A	Fulton C.I. No. 3327	Manhattan, Kan.	8.0
9F206 B	Kanota C.I. No. 839	" "	8.2
9F206 C	Brunker C.I. No. 2054	" "	6.0
9F206 D	Columbia C.I. No. 2820	" "	7.5
0F142 A	Iowa 444	East Lansing, Mich.	6.5
0F142 B	Huron	" " "	7.0
0F142 C	Markton	" " "	6.0
0F142 D	Iogold	" " "	6.0
0F142 E	Worthy	" " "	6.0
0F142 F	Wolverine	" " "	7.0
0F195 A	Iowar (Iowa) C.I. No. 847	Washington, D.C.	8.8
0F195 B	Cornellian (Idaho) C.I. No. 1242	" "	5.2
0F195 C	Richland (Idaho) C.I. No. 787	" "	4.8
0F195 D	Lenroc (Idaho) C.I. No. 3205	" "	5.0
0F195 E	Silvermine (N. Dak.) C.I. No. 659	" "	5.7
0F195 F	Fulghum (Idaho) C.I. No. 708	" "	5.7

and 6.1. No significant difference could be observed between the white and yellow varieties.

The assay of 21 samples of oats (Table VI) showed the wide range of 4.8 to 10.3 γ per gram with an average of 7.2. A considerable number of samples analyzed 8.0 and above.

TABLE VII

RYE ASSAYS

Lab. No.	Name	Obtained from	Thiamin, γ per gram
9F178	Dakold	St. Paul, Minn.	5.7
0F143	Rosen	East Lansing, Mich.	4.0
0F196 A	Dakold (Nebraska)	Washington, D.C.	5.7
0F196 B	Wisped (Wisconsin)	" "	4.7
0F196 C	Rosen (Michigan)	" "	4.5
0F196 D	Balbo (Virginia)	" "	5.0
0F196 E	Rimpau (Virginia)	" "	5.0
0F196 F	Raritan (Virginia)	" "	4.5
0F196 G	Abruzzi (Virginia)	" "	4.1
0F196 H	Smooth Neck (Virginia)	" "	5.0

Ten rye samples (Table VII) gave the relatively narrow range of 4.0 to 5.7 with an average of 4.84 γ per gram. The two samples of Dakold rye, one from Minnesota and the other from Nebraska, gave the same result, whereas two samples of Rosen rye, both from the state of Michigan, differed somewhat.

Table VIII shows the assay of five buckwheats. The two rye buckwheats are considerably higher than the others, which is probably related to the fact that they represent a different species of the herb. Three millets (Table IX) averaged 7.23 γ per gram.

TABLE VIII

BUCKWHEAT ASSAYS

Lab. No.	Name	Obtained from	Thiamin, γ per gram
0F197 A	Wing Emarginatum (Virginia)	Washington, D.C.	5.0
0F197 B	Japanese (Virginia)	" "	4.7
0F197 C	Silverhull (Virginia)	" "	4.2
0F197 D	Rye-Buckwheat Sel 37 (Virginia)	" "	7.5
0F197 E	Rye-Buckwheat (Virginia)	" "	8.5

TABLE IX

MILLET ASSAYS

Lab. No.	Name	Obtained from	Thiamin, γ per gram
0F198	Yellow Manitoba proso (Colorado)	Washington, D.C.	8.2
0F211 A	Common F.C. No. 22,637	" "	7.5
0F211 B	German (Tenn.) F.C. No. 22,636	" "	6.0

Summary

From a preliminary survey of the vitamin B_1 (thiamin) content of American cereals it is possible to draw the following conclusions:

There are significant differences in the thiamin content of the various cereals.

There are significant differences in the thiamin content of various strains of the same cereal, and there are indications that regional differences may affect the thiamin content of a single strain.

In view of the spread in values which is observed in most of the cereals it is not possible to evaluate a diet or food mixture containing cereals without analysis of the ingredients.

THE NATURE OF THE LIBERATION OF BOUND BARLEY AMYLASE AS EFFECTED BY SALT SOLUTIONS [1]

MALCOLM F. DULL and RALPH G. SWANSON [2]

Chemistry Department, DePaul University, Chicago, Illinois

(Received for publication November 1, 1940)

The early observations of a phenomenal increase in the quantity or activity of amylase enzymes during barley germination in the production of malt have since been repeatedly confirmed. Further, the nature of this increase in activity of the amylase enzymes has been the subject of much speculation and investigation. Early observers thought that it was due to a production of new enzymes, that is, synthesis. Subsequent, however, to the important discovery of latent or bound enzymes by the English investigators Ford and Guthrie (1908), this trend in thought underwent change.

Although the literature contains no specific and practical definition of the concept of a bound or latent amylase, it can be assumed from the works of various investigators that the expression refers to that portion of the total quantity of amylase present in a given barley or malt sample which cannot be extracted by distilled water. In other words, it is the quantity of enzyme that represents the difference between the "total" quantity as extracted by special methods and the "free" quantity that may be extracted by distilled water.

While at times the literature has used the terms "bound" and "latent" interchangeably, it is felt that in view of the controversial

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[1] Condensed from a portion of a thesis submitted by Ralph G. Swanson to the faculty of the Graduate School of DePaul University in partial fulfillment of the requirements for the degree of Master of Science.

[2] Present address: Research Division, Siebel Institute of Technology, Chicago, Illinois.

nature of the subject the terms should be qualified. The latent and bound amylases may be understood as those which are inactive, as evidenced by their inability to catalyze their respective characteristic reactions. This inability may be due to either physical or chemical causes, according to prevailing controversial theories. If the inability is due to physical causes, wherein the surface of the enzyme is thought to be mechanically isolated from the sphere of activity by effects of adsorption or by enveloping protein films, the enzyme may conveniently be designated as a "bound" enzyme. If, on the other hand, the inactivity is due to chemical causes, wherein the enzyme is thought to be restrained by chemical inhibitory effects and rendered dormant, or the dormant enzyme is thought to be stimulated by chemical activatory effects and rendered active, the enzyme may be designated as a "latent" enzyme. As will be evident in this paper, salt solutions liberate "bound" amylases from barley.

Since the presence and quantity of enzyme is manifested only by the extent of its activity, the bound or latent enzyme must be released from its inactive state and brought into a condition of activity in order that its presence may be detected and its quantity measured. In order to effect this release, special methods of extraction must be employed. The special methods used by various investigators in the extraction of the bound or latent amylase involve the use as extraction media of solutions of salts, peptone, proteolytic enzymes such as papain and pepsin, and such reducing substances as hydrogen sulphide and hydrocyanic acid. Each investigator advances his own interpretation regarding the mechanism of the liberation of the inactive enzyme through the presence in the extraction medium of any of the specific substances mentioned. The subject of this work, however, is the nature of the liberation of inactive amylases by only one of the specific substances mentioned, namely, salts.

Résumé of Literature

More than a decade before the importance of pH in relation to enzymic activity and the dual aspect of the diastatic enzymes were recognized, Ford and Guthrie (1908) made the important observation that the addition of salts, proteolytic enzymes, or certain other substances to barley meal increased the diastatic activity of the meal. They explained the increase in diastatic activity caused by the addition of salts by the assumption that the enzymes were protected from destruction which would otherwise occur in the absence of salts. The protection of the enzymes was believed afforded by accompanying substances previously present, but put into solution by the added salts.

The observation of the increase in diastatic activity by Ford and Guthrie was confirmed in its essentials by a number of later investigators.

The work of Ford and Guthrie was reinvestigated and elaborated by Józsa and Gore (1932), who examined the increase of the saccharifying and particularly the liquefying activity in wheat flour, malt, and barley, caused by the presence of added salts and proteolytic enzymes in the extraction media. These authors believe that the presence of salts in the extraction medium brings about the observable increase in diastatic activity by increasing the solubility of the liquefying and saccharifying enzymes. Since the influence of the salt becomes less pronounced with increasing salt concentration in the extraction medium, this action was thought by the authors to be a purely physical one. This may be interpreted as indicating a bound condition of the enzymes.

Chrzaszcz and Janicki (1933) have developed an elaborate theory to explain the increase of amylase activity which is exhibited during barley germination or through the use of special extraction methods. They believe that a latent form of amylase exists in barley which is rendered active by substances formed during germination. According to these authors, there are two substances in germinating barley, an inhibitor and activator, called sisto-amylase and eleuto-amylase respectively. With progressive protein degradation, increasing quantities of eleuto substances are formed, leading to the stimulation of the latent enzyme. The sisto substance, it is believed, has no destructive action on the amylase but merely inactivates it through adsorption or by the formation of a complex association product. Both the sisto-amylase and the eleuto-amylase accumulate during germination of barley, the latter counteracting the former. According to these authors, the eleuto substances could be neutral salts like NaCl or could be protein degradation products like peptone. Hind (1938) points out that the above authors' "conception of the development of enzyme activity is not universally accepted and the present state of uncertainty in regard to the nature of diastase is made more apparent by the suggestion that its activity is determined not only by the presence of activators but, more particularly, by substances which inhibit its action." DePlanque (1936) in elaborating upon the sisto-eleuto system of Chrzaszcz and Janicki, compares the action of salts as eleuto substances to that of peptone. Thus when peptone is added to the malt or barley infusion there is an increase in soluble protein that is foreign to the system, while with the addition of salt there is an increase of soluble protein that is native to the system.

Following is the experimental work carried out for the purpose of extending the interpretations of certain investigators whose opinions have been referred to above.

Experimental

In view of the fact that the amylases of wheat flour are more rapidly and completely extracted by neutral salt solutions than by water alone, the possibility suggested itself to Blish, Sandstedt, and Kneen (1938) that the amylase is most highly concentrated in the globulin fraction, and Hopkins and Krause (1937) indicate that the inactive barley amylase is bound to the hordein fraction. Myrbäck and Myrbäck (1936) believe that the inactive amylase may be completely liberated from the protein, to which it is thought to be bound, by means of proteolysis. Bayliss (1925) states that the solubility of certain enzymes is closely related to the dispersive power of the solvent. In this connection, it may be thought that the greater the dispersive power of the solvent (dispersion medium) the greater will be the quantity of amylase colloidally dispersed from the particles of insoluble matter (proteins) to which they possibly are adsorbed or bound. If the bound amylase is adsorbed on proteins, it is possible that the dispersion of these proteins would bring about the liberation of a portion of the bound amylases. Gortner (1937) has shown that the degree of dispersion or peptization of proteins of cereals varies with the concentration of the salt solution and with the nature of the salt. The following experiments were designed to determine whether there may be a relationship between the peptization of proteins and liberation of bound amylase.

According to Dull and Swanson (unpublished data) the apparent maximum quantity of bound amylase is liberated by approximately $0.1M$ salt solutions. Concentrations of salt greater than $0.1M$ have a depressing effect on the diastatic activity. In order to measure the quantity of bound amylase liberated by salt solutions of concentration greater than $0.1M$, a method must be employed that will avoid the error caused by the depressing effect of the salt. After considerable preliminary experimentation, a method was adopted similar to that used by Sandstedt, Blish, Mecham, and Bode (1937) in their investigations of residual amylase activity in wheat flour. By this method the salt contained in the extraction medium is washed out of the residue, upon termination of the extraction time, thereby eliminating the depressing effect of the salt. The amylase activity of the washed residue may subsequently be determined. The amount of bound amylase liberated may be computed as the difference in amylase activity in the residue of a barley sample extracted with distilled water

and that in the residue of a duplicate barley sample extracted with a salt solution.

The bound amylase liberated and the accompanying protein peptized by KCl solutions of 1%, 5%, 10%, and 20% concentrations, were determined on three 1938 American barley samples: (A) Wisconsin Pedigree 38, (B) Atlas, and (C) another Wisconsin Pedigree 38. The data are compiled in Table I. The experimental procedure is as follows:

To each of five 50-ml. capacity centrifuge tubes was added a one-gram portion of a finely ground barley sample, together with 25 ml. of extraction medium—distilled water, 1% KCl, 5% KCl, 10% KCl, and 20% KCl, respectively. The tubes and contents were kept at 20.0° C. and were shaken at 30-minute intervals during a four-hour extraction period, after which time the tubes were centrifuged, the supernatant liquid drawn off and filtered directly into a 500-ml. Kjeldahl flask. The residue, still contained in the centrifuge tube, was washed with 25-ml. portions of distilled water five times. The washing of the residue with distilled water is carried out for the purpose of completely removing the salt and of redispersing and removing the salt-coagulated proteins to effect also the removal of occluded or adsorbed amylases. After each addition of 25 ml. of wash, the contents were shaken, centrifuged, and the supernatant liquid filtered into the Kjeldahl flask. The collected filtrates in the respective Kjeldahl flasks served for the determination of the soluble protein-nitrogen, carried out in the usual manner, and calculated to milligrams of soluble nitrogen per 100 grams of sample on dry basis.

The residues in the tubes were quantitatively transferred to a 300-ml. volumetric flask with the aid of a minimum amount of ice-cold water. When each transfer was completed, the 300-ml. flask was immersed in an ice-water bath for the purpose of preventing diastatic autolysis, while the remaining transfers were being made and the necessary apparatus was being made ready to conduct the determination of diastatic activity in the residues. When the apparatus was in readiness, the 300-ml. flasks were quickly brought to 20.0° C. and 200 ml. of a 2% Lintner starch solution, buffered with sodium acetate-acetic acid buffer to a pH of 4.7, was added. The diastasis was permitted to proceed for exactly 30 minutes, after which time it was terminated by the addition of 10 ml. of 0.5*N* NaOH solution. The flasks were then made up with distilled water to capacity, and the maltose produced by diastasis was determined by the ferricyanide method of Laufer, Schwarz, and Laufer (1938), and calculated to grams of maltose produced per 100 grams of sample on dry basis.

The data, compiled in Table I, show that the extent of bound amylase liberated and the extent of protein-nitrogen peptized by salt solutions are almost parallel. This may be interpreted as a strong indication that the bound amylases, released by salt solutions, are released as a result of peptization of the proteins to which they are bound.

TABLE I

THE RELATIONSHIP BETWEEN THE LIBERATION OF BOUND BARLEY AMYLASES AND THE PEPTIZATION OF PROTEINS AS EFFECTED BY KCl SOLUTIONS OF VARYING CONCENTRATION

	Extraction media				
	Dist. H_2O	1% KCl	5% KCl	10% KCl	20% KCl
A. 6-ROW WISCONSIN PED. 38 BARLEY (FREE ACTIVITY—325)					
Amylase activity [1] in residue	38	21	15	13	10
Bound amylase liberated (by difference) [2]	—	17	23	25	28
Protein-nitrogen peptized [3]	265	370	400	420	425
B. 6-ROW ATLAS BARLEY (FREE ACTIVITY—210)					
Amylase activity in residue	45	20	16	14	13
Bound amylase liberated (by difference)	—	25	29	31	32
Protein-nitrogen peptized	296	409	450	463	470
C. 6-ROW WISCONSIN PED. 38 BARLEY (FREE ACTIVITY—379)					
Amylase activity in residue	97	41	30	24	19
Bound amylase liberated (by difference)	—	56	67	73	78
Protein-nitrogen peptized	255	386	405	420	429

[1] Amylase activity calculated as g. of maltose per 100 g. dry sample.
[2] Bound amylase liberated calculated as the difference in amylase activity between that found in the residue extracted with distilled water and that found in the residue extracted with KCl solution.
[3] Protein-nitrogen peptized calculated as mg. of protein-nitrogen per 100 g. dry sample.

The data also show that there is still some residual amylase activity remaining after extraction by a 20% KCl solution, ranging from 10 to 19 g. of maltose per 100 g. of sample on dry basis. The residual amylase after extraction with a 20% KCl solution is bound so securely that more effective liberating agents than neutral salts may be required for its release. Such liberating agents as proteolytic enzymes or reducing substances may effect its release in addition to the release of still larger quantities of amylase that are bound beneath the exposed surface of the particles of insoluble portions of the barley residue.

Gortner (1937) has demonstrated that 0.5*M* concentrations of KF, KCl, KBr, and KI peptized increasing quantities of proteins from barley, respectively. Consequently, to further test the hypothesis that the liberation of bound amylase is due to the accompanying peptization of the proteins to which the enzymes are thought to be adsorbed or bound, the following experiment was carried out. The procedure is similar to that of the previous experiment, but uses as extraction media

0.5*M* solutions of KCl and KBr. The data obtained on two barley samples, (A) 1938 Wisconsin Pedigree 38, and (B) 1938 O. A. C. 21, and on one kilned malt sample (C) of 1938 Manchuria barley, are given in Table II. These data also show that there is a strong possi-

TABLE II

THE RELATIONSHIP BETWEEN THE LIBERATION OF BOUND AMYLASES AND THE PEPTIZATION OF PROTEINS AS EFFECTED BY 0.5*M* SOLUTIONS OF KCl AND KBr

	Extraction media		
	Dist. H_2O	0.5*M* KCl	0.5*M* KBr
A. 6-ROW WISCONSIN PED. 38 BARLEY (FREE ACTIVITY—320)			
Amylase activity in residue [1]	41	24	20
Bound amylase liberated (by difference) [1]	—	17	21
Protein-nitrogen peptized [1]	261	395	420
B. 6-ROW O. A. C. 21 BARLEY (FREE ACTIVITY—365)			
Amylase activity in residue	57	26	26
Bound amylase liberated (by difference)	—	31	31
Protein-nitrogen peptized	278	412	430
C. KILNED MALT OF 6-ROW MANCHURIA BARLEY (FREE ACTIVITY—505)			
Amylase activity in residue	55	30	25
Bound amylase liberated (by difference)	—	25	30
Protein-nitrogen peptized	660	832	880

[1] See Table I (footnotes).

bility that the extent of liberation of bound amylase by salt solutions is related to the extent of peptization of the proteins. However, this relationship as presented in Table II is not strictly parallel. Only by running a large number of these tests can the correlation be definitely established. The amount of protein-nitrogen peptized by the 0.5*M* KBr was always greater than that peptized by 0.5*M* KCl, and to this extent was in agreement with the results of Gortner.

The slight lack of parallelism may be due to the possibility that protein particles are not alone in their ability to adsorb amylases, that the starch granules of barley and malt may play a role, however small, in the adsorption of the amylases. This possibility is evident from the work of Holmbergh (1933), who experimented with alpha- and beta-amylase of malt, and the starches of rice, and has shown that the amylases can be adsorbed on starch granules.

Summary

Experimental evidence is presented that indicates that the bound amylase of barley, as released by salt solutions, is released as a result of the peptization of the proteins to which the enzyme is thought to be adsorbed or bound.

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